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AN EXAMINATION OF THE ACTIVE CONSTITUENTS

OF

SENNA LEAVES AND PODS

- by -

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CONTENTS.

AN EXAMINATION OF THE ACTIVE CONSTITUENTS OF SENNA LEAVES AND PODS.

OBJECT OF RESEARCH.

Although the medicinal use of Senna Leaves and Pods has been known since the 10th century, when they were used by the Arabian physicians, the nature of their constituents is still in some doubt. A number of investigators have studied the constituents of the leaves during the past 50 years, but little work has been done on the fruits, and even the British Pharmaceutical Codex 1934, a standard reference book, states that "Senna fruit appears to contain the same active constituents as the leaf" - a rather indefinite statement.

The leaves and fruits of two different varieties of Senna are used in medicine, and are official in the British Pharmacopoeia 1948, viz.:

(a) Alexandrian Senna, obtained from Senna acutifolia.

(b) Tinnevelly or Indian Senna, obtained from Senna angustifolia.

The present investigation was carried out in order to attempt to elucidate the following problems:

1. To find a simpler method of isolating the active constituents of Senna Leaves and Fruit than has so far been described in the literature.

2. To confirm, or otherwise identify, the chemical nature of these constituents, and to synthesise them.

It was realised from the beginning that the aim was ambitious and some months of experimentation showed that much fundamental research was necessary before the problem could be successfully attacked. In particular, it was found that little is known about the chemical changes which occur when anthraquinones and the anthrones are subjected to chromatographic adsorption; the lack of precise information on the reduction of substituted anthraquinones to anthrones; and the unreliability of the synthetic methods for the preparation of many hydroxyanthraquinones. Especially disappointing was the lack of reliability of many of the papers dealing with the substituted anthraquinones and one can only conclude that the investigators were concerned more with the practical applications of their substances (dyes, etc.) than with their purity and that much of their work was hasty and incomplete. It was incidentally consoling to learn through private sources that other workers in the field have recently come to the same conclusion. The need for reliable data came from a paper by Gibson and Schwarting (1) who found that they had to conduct comparative experiments with pure compounds before they could differentiate the zones in a chromatographic examination of cascara bark.

In view of the above, it was decided that the

thesis must be largely exploratory and the investigation was therefore carried out in three parts:

1. Chromatographic examination of senna extracts.
2. Synthesis of anthranols by reduction of anthraquinones.
3. Synthesis of hydroxyanthraquinones.

INTRODUCTION.

1. Active Constituents of Senna.

Tschirch and Hiepe (2) attributed the purgative properties of the leaves to hydroxy-methyl-anthraquinone derivatives, Alexandrian containing more than Tinnevelly, while the fruits contained more than the leaves. The substances isolated by these workers were cathartic acid (which on hydrolysis yielded senna-rhamnetin, m.p. above 260°C), anthraglucosennin, glucosennin $C_{22}H_{18}O_8$, m.p. above 260°C, senna-emodin $C_{15}H_{10}O_5$, m.p. 223-224°C, senna-iso-emodin $C_{15}H_{10}O_5$, senna-rhamnetin, senna-chrysophanic acid, and an unnamed substance $C_{14}H_{10}O_6$.

Tutin (3) carried out exhaustive researches on the leaves, and isolated the following substances from Tinnevelly leaf:

rhein, kaempferol, aloe-emodin, kaempferin $C_{27}H_{30}O_{16}$ (m.p. 185-195°C) a new glycoside of kaempferol, a mixture of glucosides of rhein and aloe-emodin.

He found the Alexandrian leaf to contain the same substances together with iso-rhamnetin, both free and as a glucoside. He could not confirm the statements of Tschirch and Hiepe that "senna-emodin", "senna-chrysophanic acid" and a substance "$C_{14}H_{10}O_6$" were present. He maintained that the anthraquinone derivatives present consisted solely of rhein and aloe-emodin.

No further references could be found until that of Straub and Gebhardt (4) who stated that the active

constituents of the leaf (variety not named) are anthranol glycosides, and when these are hydrolysed by acid the anthranol is readily changed to quinone. A pure crystalline glycoside was obtained which was very difficult to hydrolyse with acid. Another glycoside fraction, which could not be obtained pure, was hydrolysed by quite weak acids, such as the vegetable acids in the leaf itself. The leaves are said to contain about 1% of the latter glycoside, which is stated to be the principle active constituent.

Triende (5) found that the active substance in the leaf (variety not stated) is a very stable anthranol glycoside. It can be split into its components only by boiling with 10% hydrochloric acid. Fermentative decomposition of the glycoside resulted in formation of emodin, and the intermediate product, anthranol, was detected. It was concluded that senna glycoside is a β-glycoside.

Straub and v. Bergmann (6) stated that Senna leaf (variety not named) contains an anthranol glycoside which is difficult to hydrolyse with hydrochloric acid, a glycoside easily hydrolysed by hydrochloric acid, and free emodin. The last two substances are said to be inactive therapeutically. It was stated that the leaf contains 0.8% of anthranol glycoside.

Stoll, Kussmaul and Becker (7) isolated two crystalline glycosides - Sennoside A and Sennoside B - from the leaves and fruits. They were fractionally precipitated from methyl alcohol by

means of alkaline earth hydroxides. Sennoside A was found to be slightly soluble in methyl alcohol and very resistant to hydrolysis with acid, while Sennoside B was easily soluble in methyl alcohol and was hydrolysed even under mild conditions. Both Sennosides corresponded to the formula $C_{21}H_{20}O_{10}$, and both had an aglycone content of 62%. This agreed with 1 molecule of glucose in combination with an anthracene residue. The aglycone on oxidation with chromic acid under mild conditions yielded rhein. The glycosides were therefore believed to be formed from glucose and the anthranol of rhein, the difference between the two being a position isomerism of the glucose at different hydroxyls of the adjacent aglycone.

The amount of methylanthraquinones present in Senna, was stated by Maurin (8) to be

Alexandrian Leaf	wild 1.55%	cultivated 1.60%
Tinnevelly Leaf	1.35%	1.30%.

The fruit was stated to contain slightly less than the leaves.

Fairbairn (9) has recently confirmed that the activity is due mainly to anthranol glycosides.

From the above summary it is concluded that Senna leaves and fruit probably contain two anthranol glycosides, one of which is strongly resistant to hydrolysis by acids, the other being easily hydrolysed.

The chromatographic examination of vegetable drugs is only now beginning to receive detailed study, and so there are few references to this

method as applied to Senna or other anthraquinone containing drugs. Ernst and Weiner (10) used a column of magnesia, and stated that, because of its alkalinity, it had the advantage of causing the emodin containing layer to be coloured red. The whole of the lower part of the column was pale yellow, due, it was stated, to the presence of anthranols. This lower zone had a greenish yellow fluorescence in ultra violet light.

Gibson and Schwarting (1) in a recent paper on the Chromatographic Isolation of the Trihydroxy-methylanthraquinones of Cascara Sagrada used a mixture of 3 parts of Celite and 1 part of magnesia as the adsorbing agent. They had difficulty in differentiating the zones due to anthraquinones, and therefore carried out comparative experiments using dilute chloroform solutions of the pure anthraquinones which they anticipated would be present. On chromatographing a chloroform solution containing the three pure substances, emodin, aloe-emodin and iso-emodin, continued washing of the column with chloroform over a period of a week was required before differentiation of the initial red layer was evident. By preparing chromatograms of the individual substances identification of each zone became possible, and comparison was carried out with a chromatogram prepared from a chloroform extract of cascara bark. Elution of the several layers was accomplished by treating the dry powder with 10% hydrochloric acid and shaking out with chloroform. The chromatograms from the cascara

extracts showed a pale yellow layer directly below the adsorbed anthraquinones. Ernst and Weiner's belief that this yellow zone was due to anthranols was not confirmed.

Cropper (11) recommended Magnesium Carbonate "Pond" as an adsorbent for hydroxyanthraquinone derivatives.

The test usually used to detect the presence of anthraquinone derivatives in drugs is based on the fact that most natural hydroxyanthraquinones give a red colour with alkali. The test is frequently known as Borntrager's reaction as it was used by him in the identification of Aloes (12), although it appears to have been in use since 1814 (13). Fairbairn (14) has suggested modifications to the original test which makes it possible to identify (a) free hydroxyanthraquinones, (b) combined hydroxyanthraquinones and (c) hydroxyanthranols.

(a) The drug is extracted with benzene which dissolves free hydroxyanthraquinones. The benzene solution on shaking with 10% ammonia solution imparts a pink or red colour to the aqueous layer.

(b) The drug is boiled with 10% sulphuric acid for a few minutes, the acid solution filtered off, cooled and shaken with benzene. The benzene layer on shaking with 10% ammonia produces a pink or red colour to the ammonia layer, indicating that hydrolysis with acid had liberated the hydroxyanthraquinones.

(c) The acid solution from the above is boiled with solution of hydrogen peroxide. After cooling, extraction with benzene and shaking with ammonia is carried out as before. A pink or red colour indicates that hydroxyanthranols present had been oxidised to hydroxyanthraquinones.

2. Reduction of Anthraquinones to Anthranols.

Barnett and Matthews (15) reduced 1-chloro-anthraquinone by two different methods and so produced two isomeric monochloroanthrones.

(a) A solution of the anthraquinone in concentrated sulphuric acid was reduced by the gradual addition of aluminium powder at a temperature of 20° - 30°. 1-chloro-9-anthrone was produced.

(b) A boiling solution of the anthraquinone in glacial acetic acid containing tin and a trace of platinic chloride was reduced by the gradual addition of concentrated hydrochloric acid. 4-chloro-9-anthrone was produced.

Goodall and Perkin (16) used a strong, boiling solution of stannous chloride in concentrated hydrochloric acid to reduce hydroxyanthraquinones to the corresponding anthranols. The anthraquinone, without apparent solution, was almost quantitatively converted into the anthranol.

Battegay and Hueber (17) formed anthranols by prolonged boiling in an alkaline solution to which

sodium hydrosulphite was added gradually. In order to go beyond the anthrahydroquinone they stressed the necessity of working with a big excess of alkali and having a solution sufficiently dilute to ensure that the reaction products remain in solution.

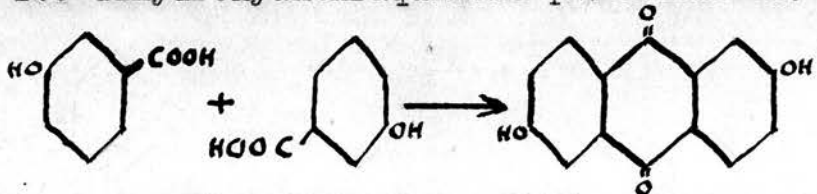
von Braun and Bayer (18) formed anthranol by hydrogenating anthraquinone under pressure in various solvents (e.g. Dekalin) at 160° - 170° in presence of finely divided nickel.

Perkin (19) used strong aqueous solution of sodium hydroxide (28-30%) and glucose to produce the reduction. The mixture was heated under pressure at 200° - 230°.

3. Synthesis of Hydroxyanthraquinones.

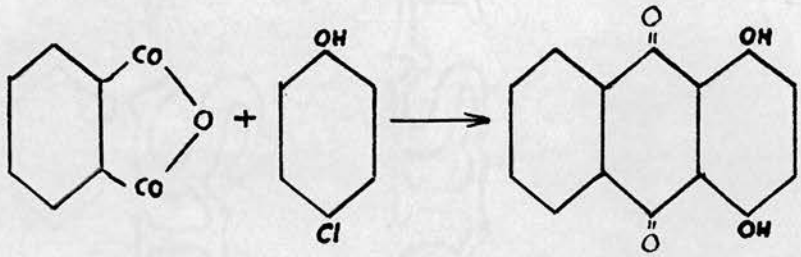
A number of general methods are known for the synthesis of anthraquinone derivatives, and these are usually applicable to hydroxyanthraquinones. Alternatively, these methods may be used to form derivatives such as halogen anthraquinones which may in turn be converted into the hydroxy-compounds.

(a) Condensation of two molecules of aromatic carboxylic acids. This method was used by Schunck and Römer (20) who produced a mixture of dihydroxyanthraquinones by heating m-hydroxybenzoic acid with 90% sulphuric acid. 2:6-dihydroxyanthraquinone predominated.



(b) Phthalic anhydride (or substituted phthalic anhydrides) may be heated with a phenol in presence of aluminium chloride (sometimes with addition of sodium chloride), sulphuric acid or boric acid, or a mixture of the last two. Waldmann and Sellner (21) condensed phthalic anhydride with o-cresol by heating with aluminium chloride and sodium chloride at 165°, thus forming 1-hydroxy-2-methyl-anthraquinone.

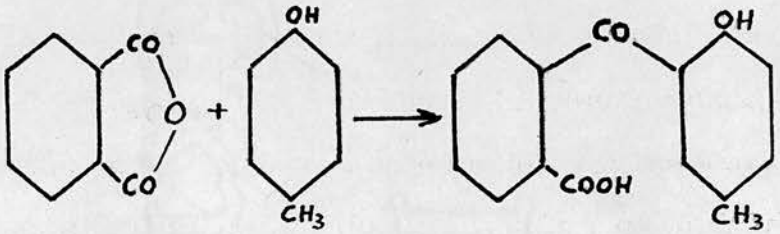
Quinizarin has been prepared by heating phthalic anhydride with p-chlorophenol at 200° in 95% sulphuric acid containing boric acid (22).



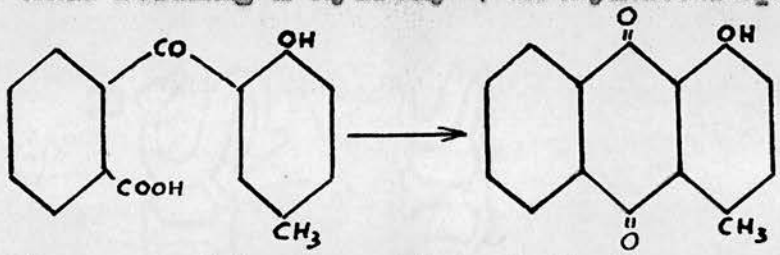
(c) A similar method to the above may be carried out in two stages. Phthalic anhydride and a phenol are heated with aluminium chloride, sulphuric acid or boric acid to form a benzoylbenzoic acid. Ring-closure of the latter is then accomplished usually by heating with sulphuric acid under carefully defined conditions.

When aluminium chloride is used, a solvent such as benzene, carbon disulphide or tetrachloroethane must be present. Ullmann and Schmidt (23) heated phthalic anhydride and p-cresol in tetrachloroethane at

115° - 130° with gradual addition of aluminium chloride until HCl gas was no longer evolved. 2'-hydroxy-5'-methyl-2-benzoylbenzoic acid was produced.



The ring-closure of the latter compound was carried out by heating with concentrated sulphuric acid for ½ hour on a water bath, thus forming 1-hydroxy-4-methylanthraquinone.



The use of boric acid in both the condensation and the ring-closure is illustrated by the preparation of 1-hydroxy-3-methylanthraquinone described by Bentley, Gardner and Weizmann (24). A mixture of phthalic acid (or anhydride), m-cresol and boric acid was heated at 170° - 180° and 2'-hydroxy-4'-methyl-2-benzoylbenzoic acid extracted from the reaction mixture. Ring-closure was effected by dissolving this acid with twice its weight of boric acid in concentrated sulphuric acid, and then adding fuming sulphuric acid carefully until the initial yellow colour changed to intense red. A number of other methods have been used for the conversion of benzoylbenzoic acids

into anthraquinones, although some are merely modifications of the above methods. Goldberg (25) used 5% fuming sulphuric acid in the preparation of 1:3-dichloroanthraquinone from 2':4'-dichlorobenzoylbenzoic acid, and heating at 155°- 160° for 2½ hours was required.

Graves and Adams (26) stated that in benzoylbenzoic acids, where there is an ortho or para directing group meta to the position in which condensation to the anthraquinone must take place, the difficulty of forming anthraquinones is especially pronounced. This is particularly true with phenol derivatives where the tendency to sulphonation prevents the condensation desired. Jacobson and Adams (27) used 7% oleum with a little boric acid in the formation of 1-hydroxy-3-methyl-4-bromo-6:8-dimethoxyanthraquinone from the corresponding benzoylbenzoic acid. They state that only traces of the anthraquinone were obtained when concentrated sulphuric acid was used, and that 20% oleum gave a smaller yield than 7% oleum.

Waldmann (28) dissolved 3:4-dihydroxy-3':6'-dichlorophenone-2'-carboxylic acid in nitrobenzene at 150° and added phosphorus pentoxide in order to produce the anthraquinone. When the product was sublimed in vacuo (after removing the solvent) the upper

sublimate consisted of red needles of 5:8-dichloroalizarin, and the lower portion of yellow needles of 5:8-dichlorohystizarin. In another paper, Waldmann (29) showed that benzoyl-o-benzoic acid could be converted to anthraquinone by boiling with benzoyl chloride and trichlorbenzene containing a trace of concentrated sulphuric acid.

(d) Anthraquinones can also be prepared from the benzoylbenzoic acids by reduction of the latter to benzylbenzoic acids, ring-closure of the benzylbenzoic acids in the usual way to form anthrones, and oxidation of the anthrones to anthraquinones.
Reduction of the benzoylbenzoic acids is usually carried out by the use of zinc, and either acetic acid, or alkali such as caustic soda or ammonia.
Ullmann (30) boiled o-benzoylbenzoic acid with 80% acetic acid and zinc strips for one hour, producing the lactone of o-benzhydryl-benzoic acid. The lactone was boiled with hydriodic acid and yellow phosphorus in a stream of CO_2 to form the benzylbenzoic acid. Scholl et alt. (31) boiled for 15 hours with zinc dust and 2N caustic soda solution, while Barnett and Wiltshire (32) carried out the reduction by heating the benzoylbenzoic acid with zinc dust and diluted ammonia containing a little copper sulphate, on a water bath for 20 hours.

Hydroxyanthraquinones may also be formed by replacement of halogen-, amino-, nitro-, and sulphonic acid groups by hydroxyl groups.

(e) Replacement of Chlorine by Hydroxyl.

Heating with fuming or concentrated sulphuric acid has been used by a number of workers to replace chlorine in the ∝-position by OH. Boric acid is sometimes added to the reaction mixture. Ullmann and Schmidt (33) used this method to convert 1-chloro-2-methyl-4-hydroxyanthraquinone to 2-methylchinizarin. Concentrated sulphuric acid and boric acid were used, the mixture being heated at 150°-160° for 3 hours.

Graebe and Liebermann (34) in their classic synthesis of alizarin from 2:3-dibromoanthraquinone fused the latter substance with caustic potash at 170°. Diehl (35) used caustic soda at 200° to convert tribromoanthraquinone to purpurin. He carried out this same reaction with tetrabromoanthraquinone, and claimed to have produced trihydroxyanthraquinone.

A milder alkali such as calcium hydroxide can be used to produce the same results. Frey (36) heated a mixture of 5:8-dichloro-chinizarin with water, slaked lime and a trace of copper, at 250° for 20 hours, and so formed 1:4:5:8-tetrahydroxyanthraquinone. Hovermann (37) showed that this method probably replaces the chlorines in the

α-position only, as using tetrachlorochinizarin he produced tetrahydroxydichloroanthraquinone.

Another method of converting chlorine into hydroxyl is described by Fischer and Sapper (38) and involves two stages. 1:4-chloromethylanthraquinone was heated at 100° for 5 hours under pressure with a concentrated solution of caustic potash in methyl alcohol, and on cooling yellow needles of 1:4-methoxymethylanthraquinone were formed. This substance was heated at 100° for 5 hours under pressure with a mixture of glacial acetic acid and concentrated hydrochloric acid, thus producing 1:4-hydroxymethylanthraquinone.

The conversion of the chlorine to methoxy- can also be carried out by heating with metallic sodium dissolved in methyl alcohol in a sealed tube for 6 hours at 160° - 180° (Keimatsu, Hirano and Yoshimi (39)).

Nitric acid can also be used to replace chlorine by hydroxyl, but it has the disadvantage of introducing a nitro- group as shown by Fischer and Rebsamen (40).

The present study did not extend to the investigation of the replacement of amino-, nitro- or sulphonic acid groups by hydroxyl.

DISCUSSION.

1. Chromatographic Examination of Senna Extracts.

Alumina was first used as the adsorbent in the chromatographic examination of senna extracts. Separation into coloured zones took place readily, but it was soon found that elution of the adsorbed substances was very difficult. Continuous extraction with an organic solvent such as chloroform or ether was necessary to elute the zones at the top of the column - and it was found that it was these zones which contained the hydroxyanthraquinones. The chromatogram from the chloroform extract had a red zone at the top, which gave a reaction for free anthraquinones. None of the lower zones gave such reaction, and no reaction was given for combined anthraquinones or for the presence of glycosides. This was as expected, as only the free anthraquinones are soluble in chloroform. Even after several hours extraction with ether the top zone still remained red, probably due to the hydroxyanthraquinones having combined with the alumina to form lakes, as mentioned by Cropper (11).

A similar column prepared from an alcoholic extract gave an olive green zone at the top which gave reactions for both hydroxyanthraquinones (free and combined) and glycosides. The second zone, which was orange, gave no reaction for anthraquinones, but did give a positive reaction in Molisch's Test. This may have been due to hydrolysis of some of the glycosides, and the free

sugars, being less strongly adsorbed than the anthraquinones, were carried lower down the column. Elution of these two zones still caused great difficulty. The ability of alcohol to extract both free and combined anthraquinones was confirmed.

In a search for a substance which would be less adsorbent towards the hydroxyanthraquinones, extracts of senna were chromatographed on columns of Magnesium Carbonate, Magnesium Oxide, Calcium Hydroxide, Calcium Carbonate, Prepared Chalk and Kieselguhr. Owing to the fineness of the powder in each case, none of these was found suitable, percolation of the liquid being extremely slow. One column was prepared using a mixture of Heavy Magnesium Carbonate 1 part and Potato Starch 3 parts. Through this was passed the chloroform eluate from the red (top) zone of an alumina column prepared from a chloroform extract. Development with methyl alcohol produced separation into three zones, the two top ones giving reactions for hydroxyanthraquinones, thus showing that at least a partial separation of these constituents had been effected. Rate of percolation was still very slow, but elution was carried out fairly simply by dissolving the magnesium carbonate in dilute sulphuric acid and extracting with chloroform in a separating funnel.

Partition chromatography using silica gel was not successful.

As alumina was the only substance readily available in the correct particle size for allowing

percolation of liquids, it was decided to reduce its adsorptive power by removing free alkali by washing with dilute hydrochloric acid and then with methyl alcohol as described by Williams (41). The alcoholic extract which was treated on this column was prepared from leaf which had previously been extracted with chloroform to remove free anthraquinones. Separation into six zones was effected, which was a greater separation than anything previously obtained. Elution, however, still presented the same difficulty, continuous extraction for several hours with alcohol in a Soxhlet apparatus not being completely effective. Zones 1, 2 and 3 gave positive reactions with Borntrager's test, while zones 2, 3, 4 and 5 gave positive reactions with Molisch's test. This indicated that partial hydrolysis had occurred.

As the attempt to reduce the adsorptive power of the alumina had not produced the desired effect, it was decided to acetylate the hydroxy- groups in the anthraquinones so as to prevent them reacting with the alumina. The solvent was removed from an alcoholic extract of the leaf, and the residue was acetylated with acetic anhydride in the usual manner. An acetone solution of the acetylated product was chromatographed on neutral alumina. Six zones were formed, but elution was again very difficult, solvents such as chloroform, ethyl alcohol and methyl alcohol having no effect. It was found that repeated extraction with hot acetic anhydride was necessary. The quantities of

material obtained from the extraction of the various zones were so small that further investigation of them was not considered.

Acetylation was carried out on two more extracts: (a) a chloroform extract, and (b) an alcoholic extract prepared from leaf previously extracted with chloroform. In both cases the same difficulty was experienced when attempting elution.

The chromatography of known substances, similar in nature to the constituents of Senna, was now considered. A solution of 1:8-dihydroxy-anthranol in benzene was chromatographed on a column of alumina. The main zone, which was orange (orange-red in ultra violet light) was bordered above and below with narrow zones indicating impurities. The main zone, even after extrusion, was unaffected by hot chloroform, alcohol or pyridine. A similar result was obtained when the anthranol solution was chromatographed on heavy magnesium carbonate.

An acetone solution of the diacetate of 1:8-dihydroxyanthranol (m.p. 208° - 209°) was chromatographed on neutral alumina. Again, development and elution proved very difficult. When a solution of the same substance in chloroform was chromatographed on heavy magnesium carbonate, elution was effected by continuing to pass chloroform through the column, and the filtrate on evaporation gave the original substance (m.p. 208°- 210°) checked by mixed melting point.

It was thus proved that acetylation of

dihydroxyanthranol reduced the firmness with which it is adsorbed on magnesium carbonate and thus renders it suitable for chromatographing on that substance.

According to Rosenthaler (42) aloin is a glycoside whose aglycone is aloe-emodin-anthranol which is combined with arabinose. It is thus very similar to the Senna glycosides described by Straub and Gebhardt (4). The aglycone may be identical, but the sugar present in the Senna glycosides is stated to be glucose. The similarity between the compounds, however, suggests that their adsorptive properties may be very similar. A 1% solution of aloin in methyl alcohol was chromatographed on neutral alumina. Elution was obtained very slowly with methyl alcohol and the filtrate on evaporation gave unchanged aloin. This method was carried out on a very small scale, only 5 ml. of solution being used, and it was felt that several days would be required for elution of quantities similar to those of the senna extracts.

A similar solution was chromatographed on magnesium carbonate. Elution was fairly easy using methyl alcohol, but the filtrate on evaporation gave a reddish brown residue which did not melt below 300°. This showed that decomposition of the aloin had occurred, probably due to hydrolysis on the alkaline adsorbent, and possibly subsequent oxidation. An amyl alcohol solution of aloin produced the same result.

The acetate of aloin was prepared, and a

solution in chloroform was chromatographed on neutral alumina. Elution with chloroform was successful, and the original aloin acetate was recovered from the filtrate, its identity being confirmed by melting points. The use of a column of magnesium carbonate gave the same results.

The conclusions to be drawn from this preliminary chromatographic work are:

1. Alumina is too strong an adsorbent for use with hydroxyanthraquinone derivatives, probably owing to the formation of lakes. Removal of free alkali from the alumina by washing with dilute hydrochloric acid and subsequent washing with methyl alcohol, and air drying, to reduce the adsorptive power, does produce some improvement, but not sufficient to make it of practical use.
2. Heavy Magnesium Carbonate gives more promising results. The fact that it dissolves readily in dilute acids thus liberating the adsorbed substance, which may be extracted by an immiscible solvent, is very useful. Its alkalinity is a disadvantage, and may lead to decomposition of the adsorbate.
3. Acetylation of the hydroxyanthraquinone derivatives before chromatography reduces the extent to which they are adsorbed and so makes elution easier.

The difficulties which were encountered during

the chromatographic work suggested that more progress might be made in that direction if more information were available regarding the properties of anthranols and hydroxyanthraquinones. It was therefore decided to carry out syntheses of anthranols and hydroxyanthraquinones, and to gather together information which would lead to the synthesis of such compounds as are found in Senna. If this could be accomplished, the behaviour of these substances on chromatographic columns could be studied, and so act as guides to the chromatography of extracts of Senna. After this decision had been made, the paper by Gibson and Schwarting (1) became available, and it was realised that similar difficulties had been encountered by other workers, and that these difficulties had only been overcome after they had been able to prepare comparative chromatograms using pure substances.

The necessity for a study of the syntheses of anthranols and anthraquinones soon became evident, as much of the published work on these types of compounds appears to be unreliable or incomplete. Bell and Waring (43) expressed the same view when they stated that "the literature on 9:9'-dianthryl is considerable but inconsistent".

2. Reduction of Anthraquinones to Anthranols.

The work of Barnett and Matthews (15) was repeated and confirmed. 1-chloroanthraquinone when reduced with aluminium and concentrated sulphuric acid formed 1-chloro-9-anthrone. When the same substance was reduced with tin, glacial acetic acid and concentrated hydrochloric acid 4-chloro-9-anthrone was produced. Both these products were successfully purified by chromatographing a solution in benzene on neutral alumina (see page 19).

(i) Aluminium-Sulphuric Acid Reduction.

The aluminium-sulphuric acid reduction was carried out on 2-chloro, and 2-methyl-anthraquinone. In the former instance no success was obtained, and the crude product when purified chromatographically yielded almost 90% of the starting material. The reduction of 2-methyl-anthraquinone was somewhat more successful. The crude product was chromatographed on neutral alumina from a solution in equal volumes of benzene and light petroleum. When the column was developed with the same solvent, unchanged 2-methylanthraquinone was present in the first portion of the filtrate . A later portion of the filtrate gave impure anthrone m.p. 100° - 101°, equivalent to 15% yield. Barnett and Goodway (44) when carrying out the same reduction stated that 3-methyl-9-anthrone was formed (m.p. 101°) but no yield was stated.

None of the authentic material was available for identification, and to distinguish it from 2-methyl-9-anthrone (m.p. 103°).

(ii) Tin-Glacial Acetic Acid-Hydrochloric Acid Reduction.

2-chloro- and 2-methylanthraquinone were also subjected to the tin-glacial acetic acid-hydrochloric acid reduction. In neither case was any detectable quantity of anthrone produced and the product appeared to be largely the original anthraquinone.

(iii) Stannous Chloride Reduction.

Goodall and Perkin (16) used a strong, boiling solution of stannous chloride in hydrochloric acid to reduce hydroxyanthraquinones to anthrones. This method was carried out on the following compounds:

(a) 2-methylanthraquinone.

An attempt to purify the product by chromatographing a solution in benzene and light petroleum was not successful, and the substance recovered from the filtrate was crystallised from ethyl alcohol. Creamy white needles, apparently homogeneous, having a melting point of 84° - 86° were obtained. Padova (45) gave the melting point of 2-methyl-10-anthrone as 86° - 88°, while Liebermann and Mamlock (46) gave 80° - 84°. Barnett and Goodway (44), however, showed that these melting points were from mixtures, and that the true melting point of 3-methyl-9-anthrone

(or 2-methyl-10-anthrone) is 101°. The product obtained in this case, therefore, appeared to be a mixture, probably of the isomers 2- and 3-methyl-9-anthrone. A further attempt to purify this mixture chromatographically was not successful. The filtrate was collected in 10 ml. portions, but apart from the first portion (which had m.p. 153° - 165° and was probably impure 2-methylanthraquinone) no separation took place. Attempts to purify by recrystallisation from alcohol resulted in a rise in melting points, approaching the melting point of 2-methylanthraquinone. This suggested that oxidation was taking place.

(b) 2-chloroanthraquinone }
(c) 1-methylanthraquinone }

In neither of these cases was the reduction successful.

(iv) Sodium Hydrosulphite Reduction.

Battegay and Hueber (17) used sodium hydrosulphite with a large excess of alkali in very dilute solution to reduce anthraquinones to anthrones. Long boiling is necessary, and the completion of the reaction is seen when the red colour, due to anthrahydroquinone, disappears. The fact that one could be certain when the reaction was complete gave hopes of successful results. In every case, however, where this reaction was carried out, traces, at least, of an

anthraquinone, were found in the product, indicating that oxidation had taken place during working up.

(a) 2-methylanthraquinone.

The product obtained by acidification of the reaction mixture was dried in two portions. The main portion was dried by gentle heat overnight. Its colour changed from yellow to pale brown. It was not soluble in caustic soda, and on addition of sodium hydrosulphite a red colour was obtained showing that oxidation to the anthraquinone had occurred during drying This was confirmed by melting points. A small portion which was dried very carefully remained yellow and gave reactions for anthrone.

The reduction was repeated and the product was dried in a desiccator. Yellow needles (m.p. 87° - 90°) obtained by crystallisation from alcohol, and free from anthraquinones, were chromatographed on alumina. The filtrate obtained on elution, when evaporated, gave residues with melting points approaching that of 2-methylanthraquinone. A positive test for an anthraquinone was given in each case. It was therefore obvious that oxidation had taken place on the column.

(b) anthraquinone.

Similar results were obtained when

anthraquinone was treated by the same method. The product after drying gave reaction for anthraquinone.

(c) 2-methylanthraquinone.

This was treated by the same method with the modification that nitrogen was passed through the reaction mixture throughout the period of boiling. A yellowish brown product was obtained, which, although partially soluble in warm caustic soda, gave a red colour on the addition of sodium hydrosulphite to this solution, indicating the presence of the anthraquinone.

An attempt to reduce 2-methylanthraquinone by sodium hydrosulphite in acid solution was unsuccessful.

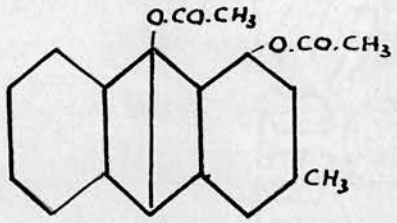
(v) Method (ii) modified by use of fuming hydrochloric acid.

The method of reduction with tin, glacial acetic acid and hydrochloric acid was carried out as before with the exception that fuming hydrochloric acid was used (S.G. 1.205 - 1.220).

The reduction of 2-chloroanthraquinone by this modification was successful. The product obtained, although the analysis was not quite accurate, agreed with the melting point of 3-chloro-9-anthrone and did not give a reaction with the "vat" test. A solution of the compound in benzene was chromatographed on alumina. Elution of the extruded column

with alcohol gave a substance which was identified as 2-chloroanthraquinone, oxidation evidently having taken place on the column. This is in contrast with the successful purification of 1-chloro-9-anthrone and 4-chloro-9-anthrone by the same method (see p. 24).

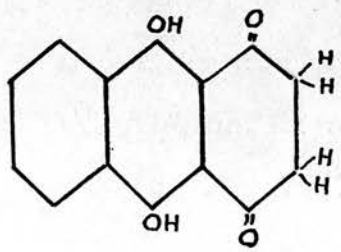
1:8-dihydroxyanthraquinone and 1-hydroxy-3-methylanthraquinone were also reduced successfully. Acetylation of 1-hydroxy-3-methylanthrone gave a compound, the analysis of which indicated that the diacetate had been formed. Analysis found C = 73.83%, H = 5.37%.



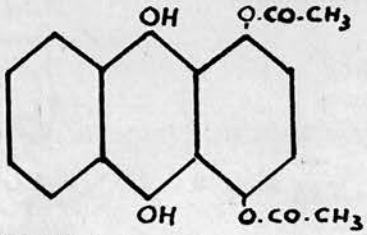
$C_{19}H_{16}O_4$ requires
C = 74.02%;
H = 5.19%.

Benzanthrone formation from this compound was not conclusive.

The reduction of 1:4-dihydroxyanthraquinone gave a product whose melting point agreed with that given in the literature for 1:4-dihydroxyanthrone (156°) but the analysis C = 69.16%, H = 4.56% was not in agreement with the calculated figures:
C = 74.33%, H = 4.42%.



1:4-dioxy-1:2:3:4-tetra-hydro-anthrahydroquinone described by Zahn (47) is possibly the compound

formed here, and its carbon and hydrogen content is in agreement with that found. (Calc. for $C_{14}H_{10}O_4$, C = 69.42%, H = 4.13%). Acetylation of this compound suggested that the diacetate  had been formed.

(vi) Stability of Anthrone.

In the sodium hydrosulphite reductions completion of the reaction (and therefore removal of the anthrone (anthraquinone) was indicated by disappearance of the red colour first formed. In spite of that, the presence of the original anthraquinone was found in the final product. 2-chloroanthrone was found to have been oxidised on a chromatographic column of alumina. It was therefore decided to investigate the stability of anthrone ($C_{14}H_{10}O$).

A sample of anthrone, free from anthraquinone, was treated by Battegay and Hueber's sodium hydrosulphite method. No red colour developed, thus confirming the absence of anthraquinone. On working up in the usual way, including the use of gentle heat for drying (about 40° - 50°) a product was obtained from which anthraquinone was separated.

Anthrone was allowed to stand in caustic soda solution for three days. At the end of that time, warming the solution with sodium

hydrosulphite gave a red colour, and anthraquinone was again separated.

When the anthrone was moistened with water and dried with gentle heat no change took place.

This proves that anthrones are unstable in presence of alkali, and are readily oxidised to the anthraquinone. The alkalinity of alumina is evidently sufficient to bring about this change in some cases. Cahn and Simonsen (48) have shown that aloe-emodin anthranol is converted to aloe-emodin by aerial oxidation in alkaline solution.

Reference page omitted?

Conclusions:

1. Heating with tin, glacial acetic acid and fuming hydrochloric acid (S.G. 1.20 - 1.22) is the best method for reducing anthraquinones to anthrones.
2. Some of the accepted methods in the literature for carrying out this reduction are unreliable.
3. Atmospheric oxidation of anthrones to anthraquinones takes place readily in presence of traces of alkali. This may occur on a chromatographic column of alumina.

3. Synthesis of Hydroxyanthraquinones.

The method adopted for the synthesis of hydroxyanthraquinones, was, in most cases, carried out in three stages.

(a) synthesis of chlorobenzoylbenzoic acids.

(b) ring-closure to form chloroanthraquinones.

(c) replacement of chlorine by hydroxyl.

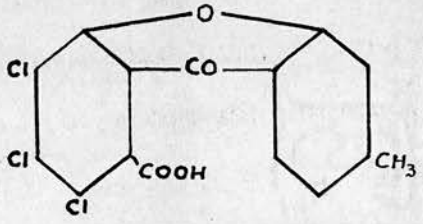
The preparation of anthraquinone-sulphonic acids was also attempted, and chloroanthraquinones formed by replacement of the sulphonic group. In one or two cases the preparation of the anthraquinone was effected without the separation of the intermediate benzoylbenzoic acid.

As an introduction to this work, the synthesis of 1-hydroxy-3-methylanthraquinone as described by Bentley, Gardner and Weizmann (24) was repeated, and the result confirmed.

(a) synthesis of chlorobenzoylbenzoic acids.

This same method was used for the condensation of tetrachlorophthalic anhydride, 3-chlorophthalic anhydride, and 3:6-dichlorophthalic anhydride, with m-cresol. In each case the operation was successful, the corresponding benzoylbenzoic acid being formed. Acetylation of the hydroxymethyltetrachlorobenzoylbenzoic acid was attempted but the analysis of the product was not in agreement with the calculated result. The hydroxymethyltetrachlorobenzoylbenzoic acid dissolved readily in sodium hydroxide solution, but immediately afterwards a white

precipitate began to form. This precipitate when separated and crystallised gave white needles, m.p. 268° - 270° with decomposition. Analysis showed the chlorine content to be 28.43%.



Trichloromethylxanthonecarboxylic acid has a chlorine content of 29.8% and the melting point in the literature is 254°. It is probable that this is the compound which was formed.

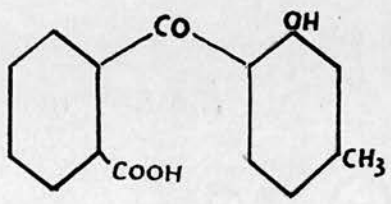
Condensation of 3-nitrophthalic anhydride with m-cresol was also attempted by the same method, but the product obtained could not be crystallised.

Ullmann and Schmidt's method (23) was used for the condensation of 3-chlorophthalic anhydride with m-cresol with successful results. This method also gave good results in the condensation of 3:6-dichlorophthalic anhydride with m-cresol, and with p-chloro-m-cresol. The yields by this method were better than those obtained by the method of Bentley et alt., and less trouble was experienced in the purification of the benzoylbenzoic acids.

(b) <u>ring-closure to form chloroanthraquinones.</u>

The numerous methods, many of which are merely modifications of one another, which

have been used to convert benzoylbenzoic acids to anthraquinones, indicate the difficulty which has been experienced in this stage of the synthesis of anthraquinones. The benzoylbenzoic acids which were treated in this investigation, all contained a methyl group in the 4' position, that is, meta to the position in which condensation would take place. Attempts were made to form anthraquinones from the following compounds:



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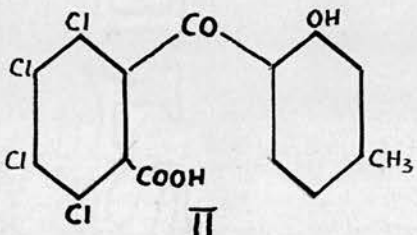

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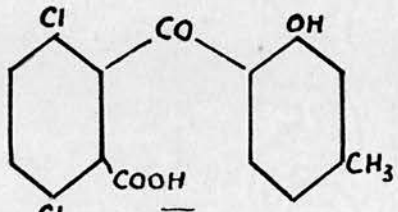
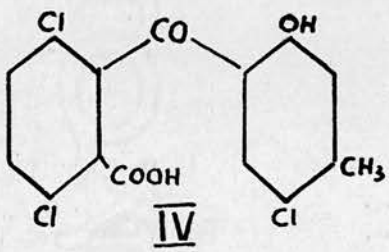


III



IV

I formed the anthraquinone without difficulty by the method of Bentley, Gardner and Weizmann (24) which involves heating with boric acid, concentrated sulphuric acid and fuming sulphuric acid. When compound II was subjected to this method no precipitate was formed when the sulphuric acid mixture was poured into water. This suggested that possibly sulphonation had occurred, and was in agreement with the findings of Graves and Adams (26) who said that anthraquinones are difficult to form where there is an ortho or

para directing group (in this case CH_3) meta to the condensing position, and that with phenol derivatives there is a tendency to sulphonation.

The ring-closure of compound III was attempted with various strengths of sulphuric acid at various temperatures. The methods of Bentley et alt. and of Eder and Widmer (49) (which was found to be successful with compound IV) were also tried. In no case was the anthraquinone formed, and sulphonation, indicated by solubility in water, appeared to have taken place in most cases. In two cases, however, white needles were obtained, which by melting point and analysis were identified as 3:6-dichlorophthalic anhydride. Thus by heating with sulphuric acid the benzoylbenzoic acid would appear to have been split into its two components.

The use of sulphuric acid under varying conditions proved unsuccessful with compound IV. The addition of boric acid to the sulphuric acid in the method of Eder and Widmer (49) resulted in the formation of the anthraquinone in good yield. It is suggested that the presence of chlorine in the position ortho to the methyl group may tend to prevent the entrance of the sulphonic acid group.

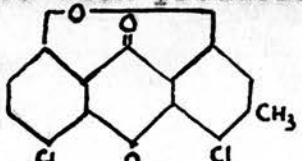
Waldmann's method (29) for converting benzoyl-o-benzoic acid to anthraquinone was confirmed. It was then carried out on

compound II. Analysis of the product suggests that hydroxymethyldichloroanthraquinone was formed.

Attempted reductions of benzoylbenzoic acids to benzylbenzoic acids were not successful.

(c) replacement of chlorine by hydroxyl.

1-hydroxy-3-methyl-4:5:8-trichloroanthraquinone was treated by the method of Ullmann and Schmidt (33), which involves heating with boric acid and concentrated sulphuric acid. The product could not be obtained crystalline, so a benzene solution of it was chromatographed on a column of magnesium carbonate. The main zone was crimson, and on elution with benzene on the column, it filtered through. This immediately suggested that the substance was not a hydroxyanthraquinone, as one would have expected such a compound to have combined with the magnesium carbonate and therefore to have been difficult to elute. After crystallising twice from benzene, the compound had a melting point of 215° - 218° and a chlorine content of 24.93%. The original anthraquinone contained 31.18% chlorine. Replacement of one chlorine by hydroxyl would have given Cl = 21.73%. If the hydroxyl originally present was eliminated, the following compound may have been produced:



This, having no hydroxyl group,

would not likely be firmly adsorbed on magnesium carbonate, and it contains 23.28% chlorine, which is somewhat near to the percentage found on analysis.

Replacement of chlorine by methoxy- was attempted by the method of Keimatsu, Hirano and Yoshimi (39). The product was not easily crystallisable, so it was dissolved in a mixture of benzene and methyl alcohol and chromatographed on alumina. Two main zones were formed - red (upper) and orange (lower). These were very difficult to elute, and after extrusion they were each extracted in a soxhlet apparatus with a mixture of glacial acetic acid and ethyl alcohol. This evidently dissolved out some of the alumina, and the sample which was analysed contained 68% of inorganic residue. The substance eluted from both zones contained chlorine, so the method was not a success.

Unsuccessful attempts to substitute hydroxyl for chlorine in 1:4:5:8-tetrachloro-anthraquinone were made by the methods of Ullmann and Schmidt (33) and Fischer and Sapper (38). Frey's method (36) proved successful, although with the surprising result that the trihydroxyanthraquinone was formed. Analysis of the compound produced gave almost identical figures for carbon and hydrogen with the theoretical figures, and the presence of only three hydroxyl groups was

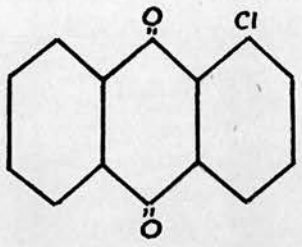
confirmed by the formation of the triacetate. This latter compound does not appear to have been prepared previously, the only reference to an acetate of 1:4:5-trihydroxyanthraquinone being the 4-monoacetate (m.p. 165°) prepared by Dimroth and Faust (50). Further confirmation that the trihydroxyanthraquinone had been formed was obtained later, when this compound was prepared from 1:4:5-trichloro-anthraquinone and a mixed melting point showed no depression.

Severe conditions are used in this method of replacing chlorine by hydroxyl. The chloro- compound is heated at 250° for 20 hours in a Carius tube with calcium hydroxide, water and a trace of copper. Investigations were carried out to determine whether any reduction in the time and/or temperature of heating would result in the formation of the tetrahydroxyanthraquinone which one would naturally expect to be formed from the tetrachloroanthraquinone. The necessity (or advisability) of using copper in the reaction was also investigated, as Ullmann (51) used copper to replace chlorine by hydrogen in 1-chloro-4-methylanthraquinone. The following modifications of Frey's method were carried out, each one having the result as shown in the table.

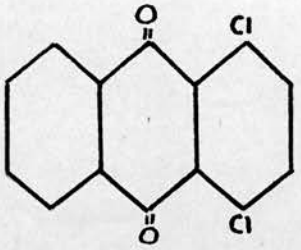
	Temp.	Time in hours	Copper	Result
1.	240°	15	Present	Trihydroxyanthraquinone, proved by acetylation.
2.	100° (reflux)	20	Present	No reaction.
3.	110°	20	Present	No reaction.
4.	150°	28	Present	Only a trace of hydroxy-compound formed.
5.	190°	16	Absent	10% alkali soluble material formed - probably trihydroxy.
6.	200°-215°	20	Absent	40% hydroxy-compound formed, proved by analysis to be trihydroxy-anthraquinone.

These experiments proved that a temperature of over 200° was required to produce a good yield, and that heating at a lower temperature for a longer period was not successful. The absence of copper did not affect the product.

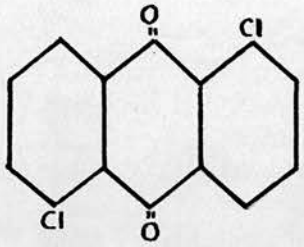
1:4:5:8-tetrachloroanthraquinone is a symmetrical molecule, so no one chlorine atom is likely to react differently from the others. In order to try and determine if any special positions of the chlorine atoms in relation to each other, resulted in the replacement of one of them by hydrogen instead of hydroxyl, the reaction was carried out on the following compounds. These compounds included all possible chloroanthraquinones with the chlorine in the α-position.



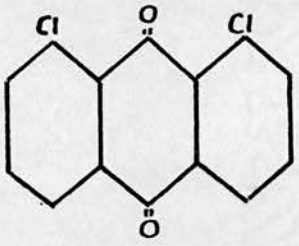
1-CHLORO-



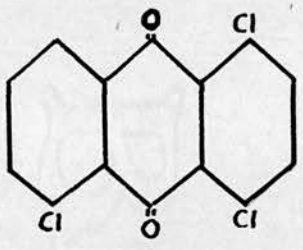
1:4-DICHLORO-



1:5-DICHLORO-



1:8-DICHLORO-



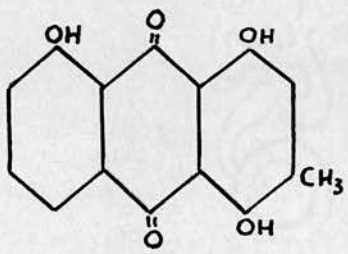
1:4:5-TRICHLORO-

In each case the corresponding hydroxyanthraquinone was formed, and it therefore appears that the replacement of one of the chlorines by hydrogen only occurs when the four α-positions are all occupied by chlorine. The only reference to this unusual reaction which could be found in the literature was that by Diehl (35) who prepared trihydroxyanthraquinone from tetrabromoanthraquinone by fusing with caustic soda. Frey produced the tetrahydroxy-compound, but had only two chlorine atoms present in his starting material (5:8-dichlorochinizarin).

The 1:4:5-trihydroxyanthraquinone formed when mixed with the compound prepared from the tetrachloroanthraquinone showed no depression in melting point thus identifying conclusively the latter compound.

1-hydroxy-3-methyl-4:5:8-trichloroanthra-

quinone was also treated by the same method in order to replace the chlorine by hydroxyl. Two substances were isolated, the analyses suggesting that one was 1:4:5:8-tetrahydroxy-3-methylanthraquinone, while the other was the trihydroxy-compound. The tetrahydroxy-compound was probably impure Cynodontin, as the melting point of the latter is given in the literature as 260° (m.p. obtained = 195° - 200°). The compound whose analysis indicated the trihydroxymethylanthraquinone had a m.p. 172° - 174°. 1:5:8-trihydroxy-3-methyl-anthraquinone has a m.p. 227°, so it is possible that the compound obtained is the other isomer:

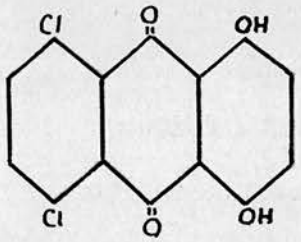


Two attempts were made to produce hydroxyanthraquinones in one stage by condensation of 3:6-dichlorophthalic anhydride with a phenol, using the method of Bigelow and Reynolds (22). These workers condensed phthalic anhydride with p-chlorophenol and produced 1:4-dihydroxy-anthraquinone.

In the first experiment toluhydroquinone and 3:6-dichlorophthalic anhydride were the reacting substances. A considerable amount of sulphur dioxide was produced during the reaction and the reaction mixture ultimately became a porous black solid, which evidently

consisted chiefly of carbon, possibly formed owing to the strong reducing action of the toluhydroquinone.

A little more success was achieved when hydroquinone was used instead of toluhydroquinone. An alkali soluble substance was obtained in small yield, which gave bronze needles on crystallising from benzene, m.p. over 300°. Analysis showed 5.87% chlorine still present. The three possible compounds are:



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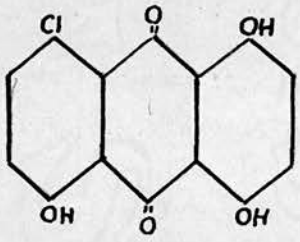

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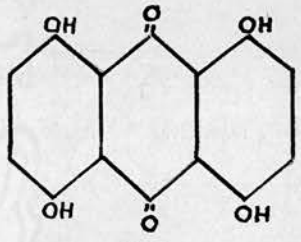


III

The calculated proportions for each of these are:

I. $C_{14}H_6O_4Cl_2$ C = 55.63% H = 1.94% Cl = 22.98%.

II. $C_{14}H_7O_5Cl$ C = 57.84% H = 2.41% Cl = 12.22%.

III. $C_{14}H_8O_6$ C = 61.75% H = 2.94%.

Found C = 56.16% H = 3.01% Cl = 5.87%.

The chlorine therefore has not been completely replaced.

Marshall (52) states that 1:4:5:8-tetrahydroxyanthraquinone melts over 300°, and gives the melting point of the acetyl derivative as 258° with decomposition. Fischer and Ziegler (53) give the melting point of the tetra-acetate as about 250° with

decomposition. The acetyl derivative which was obtained in an impure state had a melting point of 245° - 250° with decomposition, thus agreeing fairly well with the figures previously reported. It therefore appears probable that the compound produced was III - 1:4:5:8-tetrahydroxyanthraquinone, mixed with a small proportion of a chloro-derivative.

Sulphonation of Chloroanthraquinones.

The attempted preparation of 1:4:5-trichloroanthraquinone by sulphonation of 1-chloroanthraquinone (and subsequent chlorination) by the method of Goldberg (54) was not successful. Several modifications were introduced in order to try to achieve success, but none produced the desired compound. It would appear that the details of the method must be strictly adhered to, and every effort was, in fact, made to do this. There are several variables which may have been the cause of the failure.

(a) The fuming sulphuric acid used although stated to contain 20% SO_3 (and checked by S.G. 1.92) may not have been identical with that used by Goldberg. The purity of the acid to-day may be greater than that available in 1931, and some catalytic impurity, such as iron, may have then been present.

(b) The success of these sulphonations is said (55) to depend on the even distribution of the mercury catalyst throughout the mass, and the latter should be intimately mixed with the

anthraquinone used in the operation. Special care was taken with regard to this point in one of the experiments, but with no better results. A mechanical stirrer was also used.

(c) Higher temperatures may cause the sulphuric acid to act as an oxidising agent with the formation of sulphuric acid esters which give hydroxyanthraquinones on hydrolysis. A purple colour was produced on one occasion with excess caustic soda, suggesting that at least traces of hydroxy-compounds had been produced.

(d) If the yield obtained in the reaction was not the same as obtained by Goldberg then the details of quantities given by him in the working up process would not be strictly applicable to the separation of the mixture of the resulting sodium salts.

The product obtained when sulphonating 1-chloroanthraquinone with subsequent chlorination consisted chiefly of 1:5-dichloroanthraquinone. This indicated that only one sulphonic acid group had been introduced. It was therefore a surprise to find that the tetrachloroanthraquinone had been isolated when 1:5-dichloroanthraquinone was similarly treated.

1:4:5-trichloroanthraquinone was ultimately prepared by sulphonating 1:8-dichloroanthraquinone as described by Goldberg (56) and chlorinating the product. At no time did the chlorinating method produce any difficulty.

Conclusions.

1. The preparation of chlorobenzoylbenzoic acids was most successfully carried out by Ullmann and Schmidt's method (23) which uses aluminium chloride and tetrachloroethane to effect the condensation of the chlorophthalic anhydride with the phenol.
2. No general method can be recommended for ring-closure of benzoylbenzoic acids. The presence of a methyl group meta- to the position where ring-closure will take place increases the difficulty of forming the anthraquinone. The presence of a chlorine atom in the ortho- position appears to reduce the difficulty.
3. Replacement of the chlorine in α-chloroanthraquinones by hydroxyl can be accomplished by heating under pressure with calcium hydroxide and water at 200° - 215° for 20 hours. Where all four α-positions are occupied by chlorine in the original compound, three are replaced by hydroxyl and one by hydrogen.

EXPERIMENTAL.

The alumina used in the chromatographic work was Savory and Moore's "Mayfair Brand".

Melting points were determined on the apparatus described in "Qualitative Organic Chemistry" by Neil Campbell (p.7, figure 4) or on a micro-melting point apparatus (Kofler, Mikrochem. 1934, 15, 242).

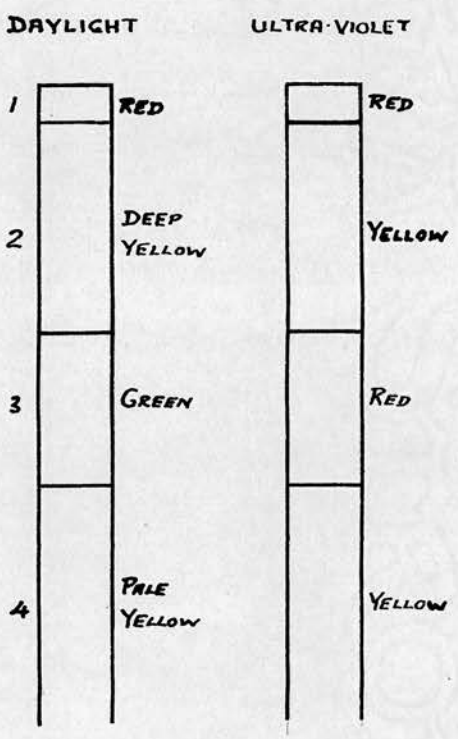
All analyses were carried out by Drs. Weiler and Strauss, of Oxford.

1. CHROMATOGRAPHIC EXAMINATIONS.

(a) Extracts of Senna.

Chloroform Extract.

10 g. of powdered Tinnevelly Senna Leaves were completely extracted with chloroform in a Soxhlet apparatus. The extract, measuring about 20 ml., was chromatographed on alumina on a column 45 cm. long and 2 cm. diameter. The results are shown in the diagrams. Development was accomplished by addition of more chloroform, the lowest layer passing completely into the filtrate.



After extrusion, the different zones were treated as follows:

Zone 1 - Red. After considerable difficulty, and the trial of various solvents, continuous extraction with ether in a Soxhlet apparatus was found to be necessary to elute the adsorbed substance. The resulting yellow solution was concentrated and allowed to evaporate. A yellow oily residue (orange in ultra violet light) containing a few crystals, remained.

This residue was found to give a negative reaction for glycosides, and a positive

reaction for free anthraquinones when examined by the following tests:

(i) Molisch's Reaction for Glycosides: To about 1 ml. aqueous solution is added 2 drops of α-naphthol in alcohol. The careful addition of 1 ml. concentrated sulphuric acid results in the formation of a violet ring at the junction of layers. On cautiously mixing by shaking under cold water a purple solution results.

(ii) Borntrager's Reaction for Hydroxyanthraquinones - as modified by Fairbairn and described on pages 8 - 9.

Zone 2 - Yellow. Easily eluted with alcohol. The solution was concentrated and allowed to evaporate spontaneously. The yellowish residue gave negative results when tested by Molisch's and Borntrager's reactions.

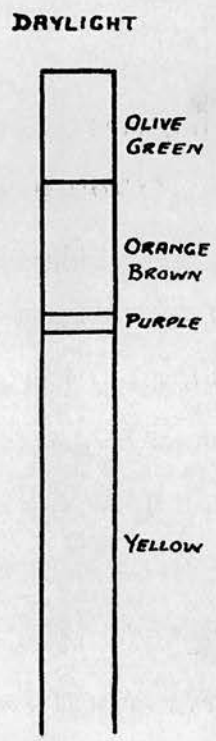
Zone 3 - Green in daylight and red in ultra violet light. These colours are characteristic of chlorophyll so this zone was not further examined.

Zone 4 - Yellow. The chloroform filtrate was concentrated and the residue allowed to evaporate. The residue gave negative reactions for glycosides and anthraquinones.

Alcoholic Extract.

10 g. powdered Tinnevelly leaf were completely extracted with industrial methylated spirit in a Soxhlet apparatus. The extract, measuring about 65 ml., was chromatographed on a column of alumina

25 cm. long and 2 cm. diameter. The result is shown in the diagram. On developing with alcohol, the yellow layer filtered through completely, but the three upper layers were strongly adsorbed. Chloroform was no more successful. On adding water, a yellow zone washed through from the upper zones and the purple zone disappeared, leaving three zones - 1 Green, 2 Orange, 3 Yellow. After extrusion the zones were examined.



Zone 1 - Green. Elution was attempted with the usual organic solvents without success, and ultimately it was necessary to extract in a Soxhlet apparatus with alcohol. A green solution was obtained which was yellowish brown in ultra violet light. After concentration and spontaneous evaporation, an oily residue remained which gave positive reactions for glycosides and also for both free and combined anthraquinones.

Zone 2 - Orange. Eluted similarly to zone 1. A yellowish oily residue containing crystals was obtained, which gave a positive reaction for glycosides but negative for anthraquinones.

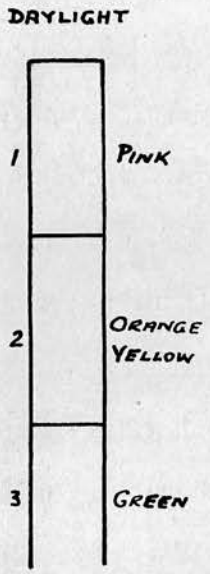
Zone 3 - Yellow. This was similarly treated. Negative reactions were obtained for glycosides and anthraquinones.

From the above preliminary experiments it was evident that the constituents to be examined were very firmly adsorbed, and long extraction in a Soxhlet apparatus was necessary to elute them from the alumina. To conduct these experiments on a larger scale would be very difficult. It was found that chloroform only extracted the free anthraquinones and that alcohol was necessary to extract the glycosidal substances. In chromatographic analysis both these solvents are considered to be strong eluents, and so it seemed difficult to get a suitable solvent to remove the adsorbed substances.

Less powerful adsorbents were tested, e.g. Magnesium Carbonate, Magnesium Oxide, Calcium Hydroxide, Calcium Carbonate, Prepared Chalk, and Kieselguhr, but all were in such a fine powder that it was only with the greatest difficulty that the liquid would percolate. Dilution of these substances with lactose or potato starch was not entirely satisfactory, although it did effect some improvement.

Magnesium Carbonate and Potato Starch as Adsorbent.

The chloroform extract from Tinnevelly leaf, prepared as formerly, was chromatographed on alumina as before. The red zone was eluted with chloroform in a Soxhlet apparatus. The eluate was chromatographed on a column composed of Heavy Magnesium Carbonate 1 part and Potato Starch 3 parts, and developed with Methyl Alcohol. Size of column 30 cm. x 12 mm. The bottom green zone filtered through.



Zone 1 - Pink. The magnesium carbonate was dissolved by dilute sulphuric acid, and the liberated adsorbate extracted by shaking with chloroform in a separator. The chloroform solution was dried over anhydrous sodium sulphate and then allowed to evaporate spontaneously. The yellow residue gave Borntrager's reaction for free anthraquinones.

Zone 2 - Orange Yellow. This was eluted with cold methyl alcohol which was allowed to evaporate. The residue gave a positive reaction for anthraquinones.

Zone 3 - Green. The filtrate was evaporated spontaneously. Borntrager's reaction was negative.

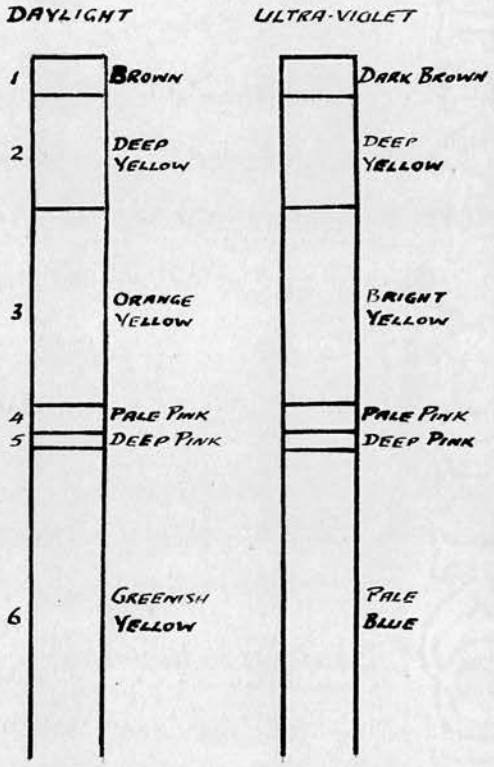
Partition Chromatography.

A column of silica gel was prepared by the method of Bell (57). The chloroform eluate from the red zone of a chromatogram similar to the above was passed through the column. No apparent separation of the constituents took place, the solution filtering through completely.

The use of neutral alumina.

The anthraquinones present in senna are probably hydroxy-compounds and are therefore slightly acidic. It was thought that the presence of free alkali in the alumina might be, at least partly, the cause of

the adsorbed substances being held so firmly. Before use, therefore, the alumina was washed with water containing a little hydrochloric acid, and then with water until the washings were neutral. In order to reduce still further the adsorptive power of the alumina, the wet material was washed several times with methyl alcohol, drained well, and air dried. This material was used for the following chromatogram.



30 g. of powdered Tinnevelly leaf, which had previously been completely extracted with chloroform (and therefore contained no free anthraquinones) were extracted with industrial methylated spirits in a Soxhlet apparatus. The resulting extract was chromatographed on a column of the alkali free alumina measuring 40 cm. x 2 cm., with the result shown in the diagrams.

On addition of more alcohol, partial development took place, and the lowest zone filtered through.

After extrusion, the zones were treated as follows:

Zone 1 - Brown. Eluted by continuous extraction with methyl alcohol. The solution was concentrated and allowed to evaporate

spontaneously. A yellow residue remained containing crystals.

Borntrager's reaction for anthraquinones - positive.

Molisch reaction - negative.

Zone 2 - Deep Yellow. Treated as for Zone 1.

Yellow residue.

Borntrager's reaction for combined anthraquinones - positive.

Molisch reaction - positive.

Zone 3 - Orange Yellow. Treated as for Zone 1.

Yellow residue.

Borntrager's reaction - positive (slowly).

Molisch reaction - positive.

Zone 4 - Pale Pink. Eluted by continuous extraction with ethyl alcohol and spontaneous evaporation. Yellow residue.

Borntrager's reaction - negative.

Molisch reaction - positive.

Zone 5 - Deep Pink. Treated as for Zone 4.

Yellow residue.

Borntrager's reaction - negative.

Molisch reaction - positive.

Zone 6 - Greenish yellow. Filtrate was concentrated and allowed to evaporate spontaneously. Greenish yellow residue.

Borntrager's reaction - negative.

Molisch reaction - negative.

Although the use of this neutral alumina gave separation into a larger number of zones than anything previously obtained, the elution of these

zones still presented considerable difficulty. Many hours continuous extraction in a Soxhlet apparatus was necessary. In order to try and reduce this great adsorbency, it was decided to acetylate the material before chromatographing.

Examination of Acetylated Alcoholic Extract.

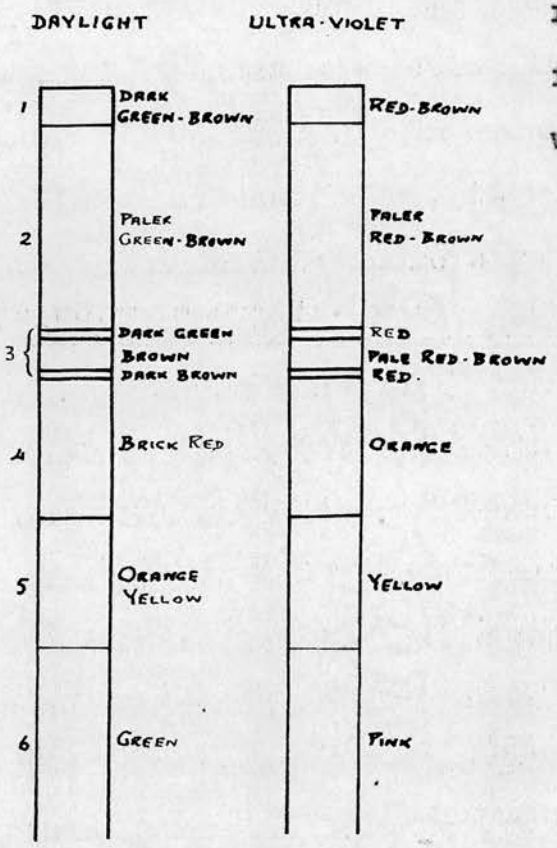
20 g. powdered Tinnevelly leaf were extracted with industrial methylated spirits in a Soxhlet apparatus until exhausted. The alcohol was distilled off, and 6 - 7 g. of residue remained. To this was added 14 ml. acetic anhydride containing 2 drops concentrated sulphuric acid, and the mixture was heated on a water bath for 30 minutes. It was poured into about 50 ml. water, warmed slightly, and the resulting precipitate filtered, washed with water, and dried.

The acetylated extract was found to be incompletely soluble in light petroleum, ether, and alcohol. Almost complete solution was obtained in acetone, so an acetone solution was prepared, filtered, and chromatographed on neutral alumina (column 40 cm. x 2 cm.).

On addition of more acetone, the lowest (green) zone filtered through.

Chloroform, ethyl alcohol and methyl alcohol were not successful as developing agents.

Elution was found to be very difficult. The method adopted was to separate the zones after extrusion and extract repeatedly with warm acetic anhydride. The solution was concentrated under reduced pressure, poured into water, and the



resulting precipitate filtered off, washed with water, and dried.

Zone 1 - Dark green.

Borntrager's Reaction - positive.

Melting Point - about 160°C.

Zone 2 - Greenish black residue.

Borntrager's Reaction - positive.

Melting Point - 110 - 115°C.

Zone 3 - Dark green.

Borntrager's Reaction - positive.

Zone 4 - Reddish brown.

Borntrager's Reaction - positive.

Zone 5 - Yellowish brown gelatinous residue, hardening on drying.

Borntrager's Reaction - negative.

Zone 6 - Dark green oily residue.

Borntrager's Reaction - negative.

Examination of Acetylated Extracts.

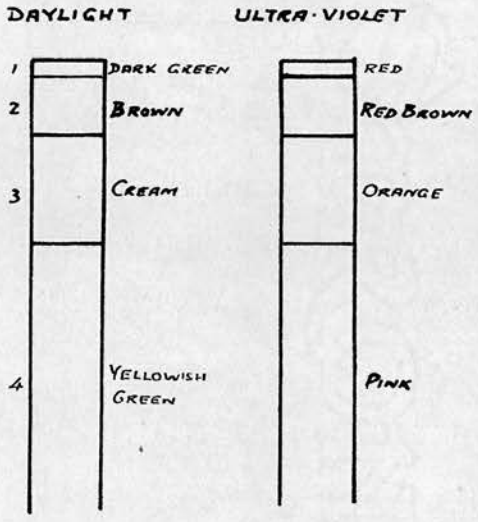
(a) Chloroform.

40 g. powdered Tinnevelly Leaf were extracted in a Soxhlet apparatus with chloroform. 7.7% of an unctuous extract remained after evaporation of the chloroform. This was heated on a water bath with 7 ml. acetic anhydride containing 1 drop concentrated sulphuric acid. The mixture

was poured into water and warmed slightly. The dark green precipitate was filtered off, washed with water and dried. The filtrate gave no reaction for glycosides nor for anthraquinones.

The acetylated substance was partially soluble in light petroleum. It was treated with this solvent, filtered, and the residue washed with the solvent. The filtrate was green in daylight and red in ultra violet, which indicated chlorophyll. It was discarded.

The dark green, washed, residue was dissolved in benzene, and chromatographed on neutral alumina, (column 35 cm. x 2 cm.).



The lowest zone filtered through.

Elution of the adsorbed substances was again difficult, and was done by extraction with acetic anhydride as described above.

Zone 1 - Dark green, softening with slight heat.
Borntrager's Reaction - positive.

Zone 2 - Dark greenish brown.
Borntrager's Reaction - positive.

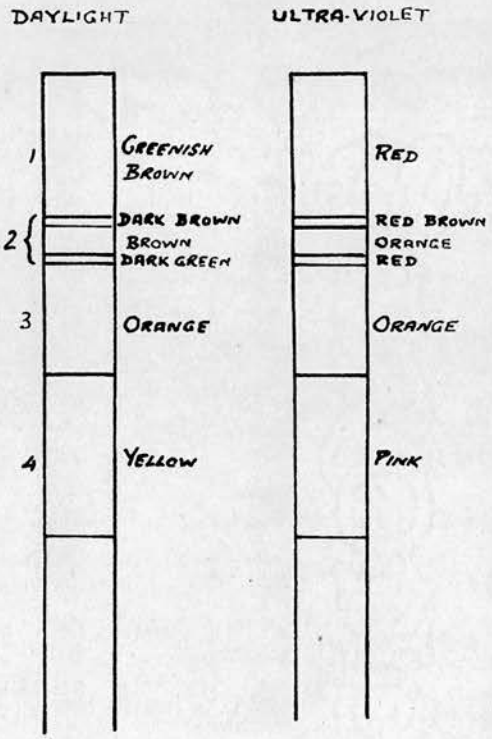
Zone 3 - Gelatinous, yellowish brown residue, similar to Zone 5 above.

Zone 4 - Yellowish green filtrate, giving no reaction with Borntrager's test.

(b) Alcohol Extract.

The powdered leaf, which had previously been extracted with chloroform, was now extracted similarly with industrial methylated spirits. After evaporation of the alcohol on a water bath, 21.2% of a sticky residue remained. This was acetylated as formerly. The filtrate gave positive reaction with Molisch's Test.

The acetylated precipitate was dissolved in acetone, and chromatographed on neutral alumina (column 35 cm. x 2 cm.).



Zone 1 - Eluted with acetic anhydride as above. A very dark, greenish black powder was obtained, which did not melt below 350°C.

Borntrager's Reaction - positive.

Molisch Test - negative.

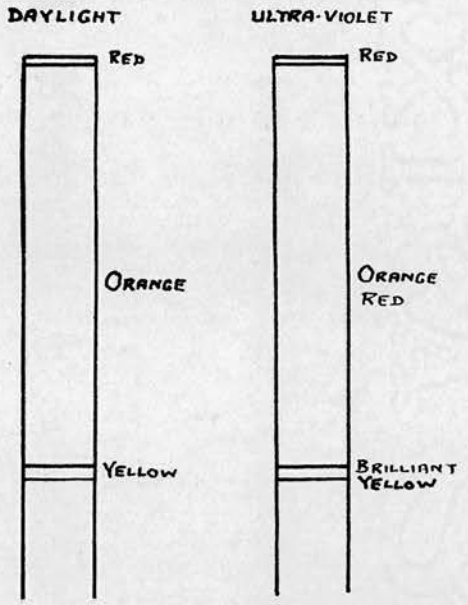
(b) Known, Related Substances.

Owing to the great difficulty experienced in development and elution of the adsorbed substances present in the various extracts of Senna Leaves tested, it was decided to carry out chromatographic experiments on known substances of a similar

constitution to those it was expected to find in Senna Leaves.

1:8-dihydroxyanthranol on alumina.

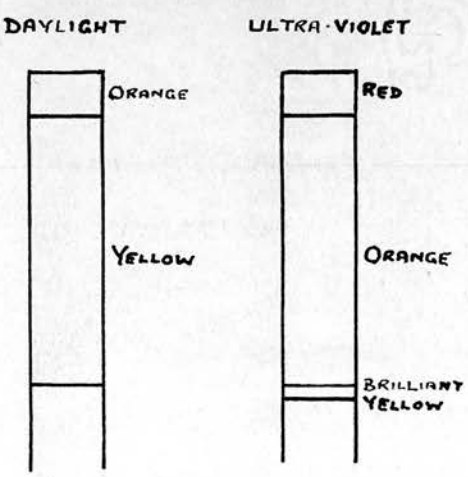
10 mls. of a 1% solution of 1:8-dihydroxy-anthranol in benzene was chromatographed on a column of neutral alumina 15 cm. x 12 mm. The solution was orange yellow in daylight, red in ultra violet.



On continuing to pass benzene through the column, the latter became yellow in ultra violet, but remained orange in daylight. Yellow colour passed into filtrate.

On changing the developing solvent to methyl alcohol, the top very narrow red layer broadened and became slightly paler. The main orange zone was very difficult to elute, and was unaffected by chloroform, alcohol and pyridine, even when warmed.

1:8-dihydroxyanthranol on magnesium carbonate.



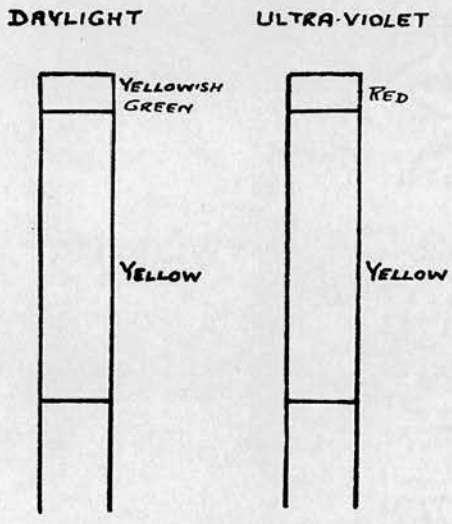
5 ml. of a 1% solution was chromatographed on heavy magnesium carbonate (column 15 cm. x 12 mm.) with result as shown. On continuing to pass benzene, the yellow

zone gradually extended the length of the column, but in ultra violet light it showed two zones, yellow and orange. Neither chloroform nor methyl alcohol gave any further development.

Di-acetate of 1:8-dihydroxyanthranol.

1 g. 1:8-dihydroxyanthranol was refluxed for ½ hour with 2 ml. acetic anhydride containing 1 drop concentrated sulphuric acid. The mixture was poured into water, filtered and crystallised from glacial acetic acid. Melting Point = 208-9°C.

(a) on alumina (neutral).



1% of the acetate in acetone was chromatographed on neutral alumina with the result as shown. No further development took place with acetone, and chloroform and methyl alcohol were similarly inactive. Acetic anhydride was more active.

(b) on magnesium carbonate.

1% of the acetate in chloroform was chromatographed on heavy magnesium carbonate. The chromatogram was similar to that on alumina. On continuing to add chloroform, a yellow filtrate passed through. This had an intensely brilliant yellow fluorescence. On allowing the solution to evaporate spontaneously a greenish brown residue was obtained which had a melting point of

208-210°C. Mixed melting point with the original substance showed no depression. M.P. of diacetate of 1:8-dihydroxyanthranol = 209-10°C.

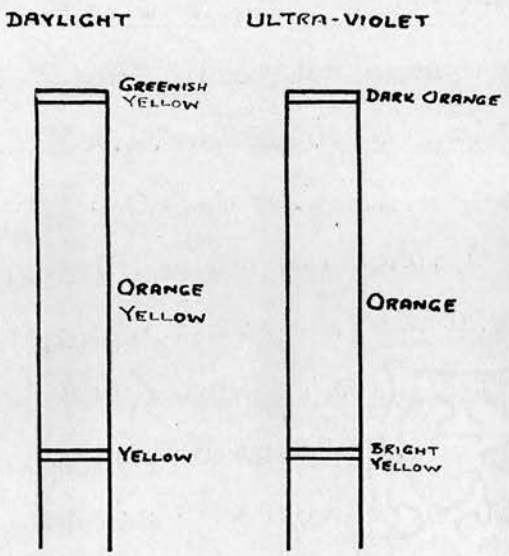
The top zone, which resisted elution, was extruded, dissolved carefully in the minimum of dilute hydrochloric acid, and extracted with chloroform. The chloroform solution was dried over anhydrous sodium sulphate, filtered, evaporated. The dark green residue did not melt below 300°C.

It was thus proved that 1:8-dihydroxyanthranol is firmly adsorbed on alumina, and on magnesium carbonate, but after acetylation, although it still has a strong affinity for alumina, it may be removed from magnesium carbonate by elution with chloroform.

Aloin on alumina.

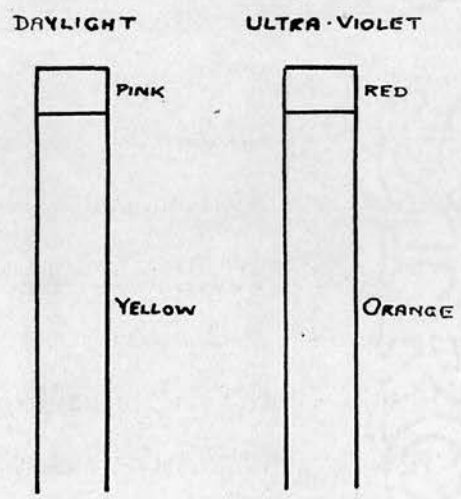
According to Rosenthaler (42) Aloin is a compound of arabinose with aloe-emodin anthranol. It is thus very similar to the Senna glycosides described by Straub and Gebhardt (4). The aglycone may be identical, but the sugar present in the latter is stated to be glucose. The similarity between the compounds, however, would suggest that their adsorptive properties would be very similar.

5 ml. of 1% aloin in methyl alcohol was chromatographed on neutral alumina (15 cm. x 12 mm.). The pale yellow solution itself was not markedly fluorescent. The chromatogram was as shown. On passing through more methyl alcohol the



fluorescence became more of a light yellow, and the middle zone increased from about 3 cm. to almost 5 cm. Further development and elution was obtained very slowly by addition of more methyl alcohol. The filtrate on concentration and evaporation gave a yellow residue of aloin.

Aloin on Magnesium Carbonate.



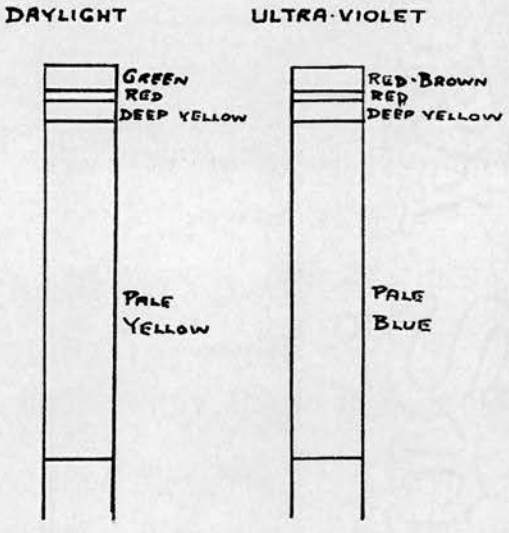
10 ml. of a 1% solution of aloin in methyl alcohol was chromatographed on a column of magnesium carbonate (15 cm. x 12 mm.) with the result as shown in the diagram. On further addition of methyl alcohol the lower zone filtered through. The filtrate, on concentration and spontaneous evaporation gave a reddish brown residue with a melting point not below 300°.

An amyl alcohol solution of aloin was chromatographed similarly, with the same results. This suggested that decomposition of the aloin took place on the alkaline adsorbent.

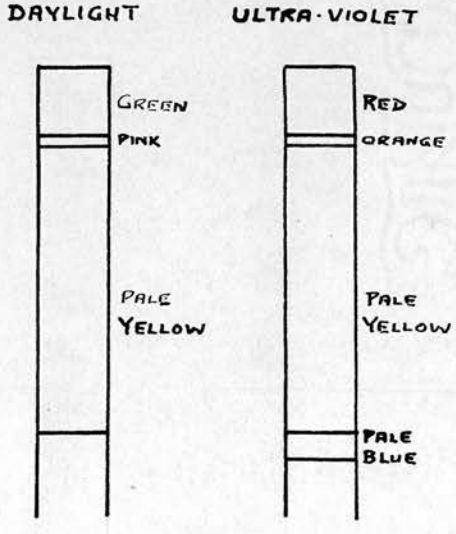
Acetylation of Aloin.

1 g. Aloin + 2 ml. Acetic Anhydride + 1 drop conc. H_2SO_4 were boiled under a reflux condenser for 30 mimutes. The product was poured into water, washed with warm water, filtered and again washed with water, and dried. 1.2 g. of a greenish yellow powder was obtained, m.p. 93-4°.

10 ml. of a 2% solution in chloroform was chromatographed on neutral alumina (20 cm. x 12 mm.) with the result shown in diagrams.



On further addition of chloroform the lowest zone filtered through as a yellow liquid with a blue fluorescence in ultra violet light. On evaporation and drying a pale yellow powder was obtained with a melting point of 93-4°. (M.P. of Aloin triacetate = 95-6°).

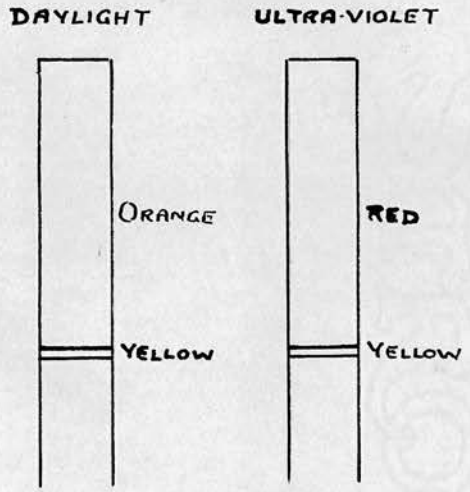


10 ml. of the same chloroform solution was chromatographed on a column (20 cm. x 12 mm.) of heavy magnesium carbonate, with the result as shown. The main yellow zone filtered through when the column was eluted with chloroform. The filtrate on evaporation and drying gave a pale yellow residue, m.p. 94° - 96°.

The top (green) zone was extruded, and the magnesium carbonate dissolved in dilute hydrochloric acid. The acid solution was extracted with chloroform. On evaporation of the chloroform solution a greenish black residue was obtained which did not melt below 300°.

Hydrolysis of Aloin.

10 g. Aloin + 10 g. Borax + 100 ml. water was boiled for 30 minutes, cooled, and made acid with hydrochloric acid. The orange red precipitate was recrystallised from toluene and gave a m.p. 195 - 200°. It complied with the tests given by Cahn and Simonsen (58) for the aglycone of aloin.

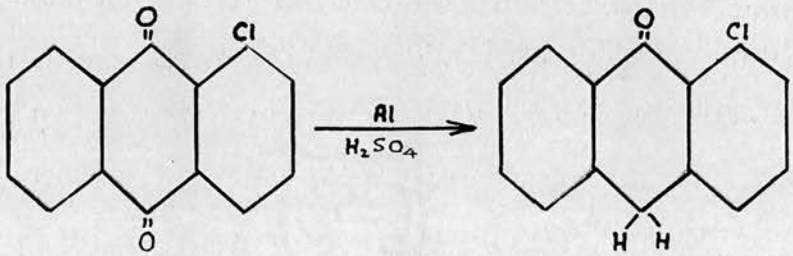


An acetone solution was chromatographed on neutral alumina, with the result shown. Elution was difficult, acetone, benzene, ethyl and methyl alcohols being of little use.

2. REDUCTION OF ANTHRAQUINONES TO ANTHRONES.

Preparation of 1-chloro-9-anthrone.

(Barnett and Matthews (15)).



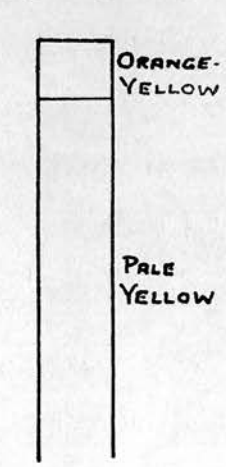
10 g. 1-chloroanthraquinone were treated as described.

Yield of crude product = 10 g.

A small portion was purified by crystallisation from a mixture of equal parts of chloroform and light petroleum (60° - 80°).

Yellow needles were obtained, m.p. 119°. The solution in methyl alcohol gave a slight blue fluorescence in ultra violet light.

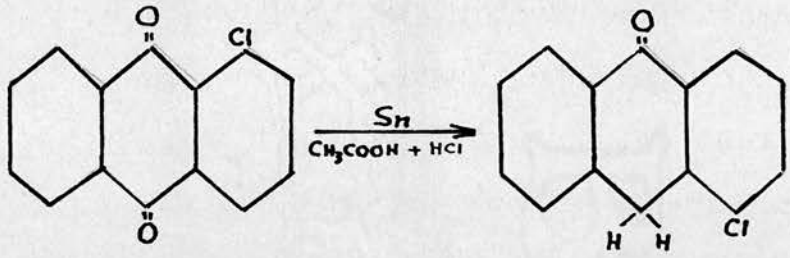
1 g. of the crude product was dissolved in benzene and filtered. Only a trace of insoluble matter was present. The filtrate was chromatographed on a column of neutral alumina (25 cm. x 12 mm.) with the result shown. No difference was observed in ultra violet light. On addition of more benzene, the pale yellow zone filtered through. The filtrate was concentrated, and allowed to evaporate. Yellow needles were obtained weighing 0.9 g., m.p. 117°. This agreed with the figure of 118° obtained by Barnett and Matthews for



1-chloro-9-anthrone. The crude material obtained by the reduction of 1-chloroanthraquinone with aluminium and conc. H_2SO_4 therefore contained 90% 1-chloro-9-anthrone.

Preparation of 4-chloro-9-anthrone.

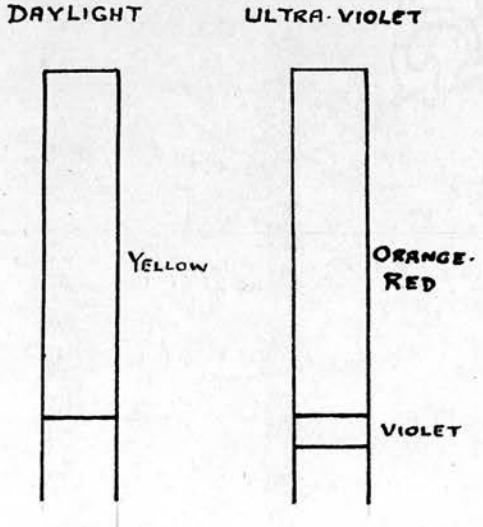
(Barnett and Matthews (15)).



10 g. 1-chloroanthraquinone, 15 g. tin, and 75 ml. glacial acetic acid containing a few drops dilute solution of platinic chloride were treated as described by the above authors.

Yield of crude product = 6.7 g.

A small portion was recrystallised twice from boiling dilute alcohol, producing yellow needles m.p. 119°. The solution in methyl alcohol gave a livid blue fluorescence in ultra violet light. This was destroyed by addition of a few drops of pyridine.



1 g. of the crude product was dissolved in benzene and filtered. The insoluble matter was negligible. The filtrate was chromatographed on a column of neutral alumina (25 cm. x 12 mm.) with the

result shown. Elution was obtained by addition of more benzene. The violet fluorescent zone was collected separately, but on evaporation yielded little or nothing. The filtrate from the yellow zone was collected separately, concentrated, and evaporated spontaneously. Yellow needles were obtained, m.p. 119°. Yield = 0.83 g.
A mixture of this substance with 1-chloro-9-anthrone had m.p. 108° - 112°, which proved that a different substance had been formed by the change in the method of reduction. The melting point agreed with Barnett and Matthews' figure of 118° for 4-chloro-9-anthrone.

The crude material obtained by the reduction of 1-chloroanthraquinone with tin and acetic acid and hydrochloric acid therefore contained at least 83% 4-chloro-9-anthrone.

The work of Barnett and Matthews has therefore been confirmed.

Reduction of 2-methylanthraquinone.

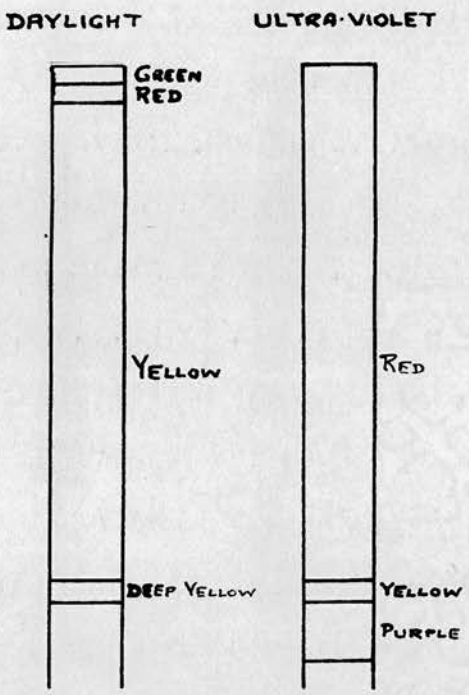
(a) By means of aluminium and sulphuric acid.

10 g. 2-methylanthraquinone were reduced with sulphuric acid and aluminium exactly as in the reduction of 1-chloroanthraquinone by that method.

The crude product was greenish yellow, had a melting range of 90 - 110° and weighed 9.5 g.

1 g. of this was dissolved in equal volumes of benzene and light petroleum (60° - 80°), filtered, and chromatographed

on neutral alumina (40 cm. x 2 cm.), with the result shown.

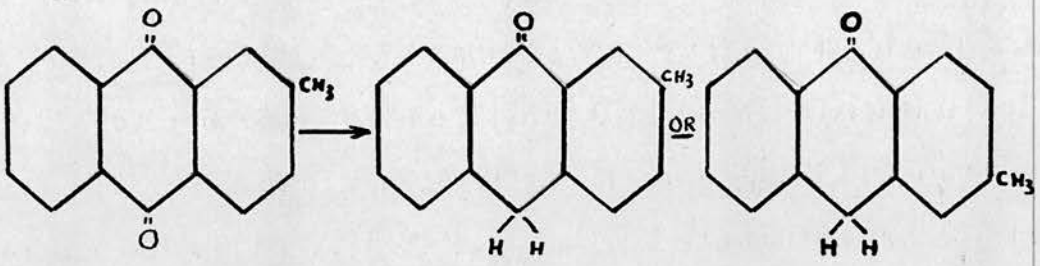


On development with the same solvent, the fluorescent purple zone passed through first, but on evaporation gave only a trace of residue.

The remainder of the filtrate was collected in 50 ml. portions.

1. Colourless. On evaporation gave yellowish white needles weighing 0.28 g. = 28% of crude substance, m.p. 174-5°.
   On mixing with 2-methylanthraquinone the melting point was unaltered. This portion therefore consisted of unchanged 2-methyl-anthraquinone.
2. Pale yellow. On evaporation gave pale yellow residue, m.p. 125 - 133°, weighing 0.19 g. = 19%.
3. Pale yellow. On evaporation gave pale yellow residue, m.p. 83 - 85°, weighing 0.15 g. = 15%.
   On recrystallisation from dilute alcohol it gave pale yellow needles, m.p. 100-1°.
   Analysis found: C 85.21%; H 5.51%.
   Calc. for $C_{15}H_{12}O$: C 86.5%; H 5.7%.
   This is evidently the impure anthrone formed by the reduction of the anthraquinone,

i.e. 2-or 3-methyl-9-one.



Barnett and Goodway (44) when carrying out the same reduction, stated that 3-methyl-9-anthrone was formed (no yield stated).

4. Pale yellow. On evaporation gave pale yellow residue, m.p. 110-40°, weighing 0.10 g. = 10%.

The remaining filtrate was yellow, and gave little residue.

This reduction has therefore yielded 15% of anthrone.

(b) <u>By means of tin, glacial acetic acid and hydrochloric acid</u>.

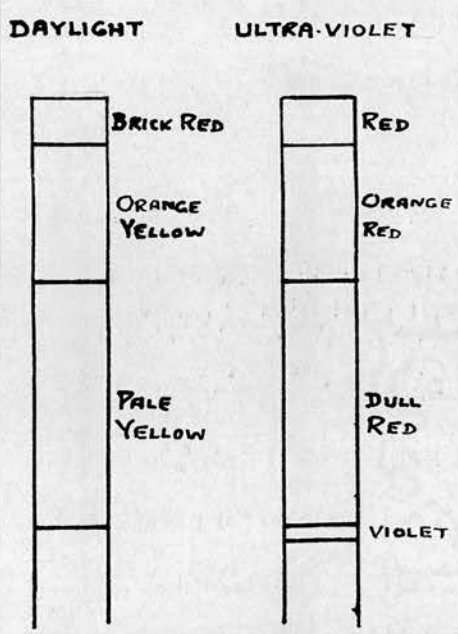
10 g. 2-methylanthraquinone were treated with tin, glacial acetic acid and hydrochloric acid exactly as in the reduction of 1-chloro-anthraquinone by that method.

The crude product, m.p. 130 - 140°, was yellowish brown and weighed 9.5 g.

1 g. was dissolved in equal quantities of benzene and light petroleum (60° - 80°), filtered, and chromatographed on neutral alumina, 30 cm. x 12 mm.

Additional benzene/light petroleum was added, and the filtrate collected in fractions:

1. 50 ml.-violet fluorescence. Residue



negligible on evaporation.

2. 25 ml.-pale yellow. No fluorescence. On evaporation gave creamy white residue, m.p. 176°, weighing 0.09 g.=9%.Mixed melting point showed it to be unchanged 2-methyl-anthraquinone.

3. 25 ml.-pale yellow, non fluorescent. Almost white residue, m.p. 175°, weighing 0.14 g. = 14%. Mixed melting point showed unchanged 2-methylanthraquinone.

4. 25 ml.-pale yellow, non fluorescent. Pale yellow residue, m.p. 160° - 170°, weighing 0.20 g. = 20%.

5. Yellow residue, m.p. 158° - 165°, weighing 0.11 g. = 11%.

Practically no further elution was obtained using the benzene/light petroleum mixture, and benzene alone was equally useless. On changing to ethyl alcohol a yellow fluorescent filtrate was obtained which on evaporation gave orange yellow crystals decomposing about 190°C., and weighing 0.2 g. = 20%.

No further elution was obtained.

This reduction has therefore not yielded any detectable quantity of anthrone, m.p. 100 - 101°.

Reduction of 2-chloroanthraquinone.

(a) By means of aluminium and sulphuric acid.

5 g. 2-chloroanthraquinone were treated with aluminium and sulphuric acid as formerly described. 4.5 g. of a cream coloured product was obtained, m.p. 170° - 195°.

1 g. of the crude product was dissolved in benzene and chromatographed on neutral alumina, 40 cm. x 2 cm. On developing with benzene, a very pale yellow filtrate was obtained which on evaporation gave 0.87 g. of pale yellow needles, which on recrystallising from equal parts of benzene and light petroleum (60° - 80°) had m.p. 200° - 203°, showing it to be unchanged 2-chloroanthraquinone.

No further elution was obtained.

The reduction was therefore unsuccessful.

(b) By means of tin, glacial acetic acid and hydrochloric acid.

5 g. of 2-chloroanthraquinone was treated with tin, glacial acetic acid and hydrochloric acid, as formerly. 4 g. of a pale yellow product was obtained which had a melting range of 130° - 220° with decomposition.

Chromatographing of a benzene solution as formerly yielded fractions of filtrate which on evaporation gave high melting residues obviously consisting mainly of unchanged 2-chloroanthraquinone. No anthrone was detected, and so the reduction was again unsuccessful.

Stannous Chloride as Reducing Agent.

(Goodall and Perkin (16)).

These workers used a strong, boiling solution of stannous chloride in hydrochloric acid to reduce hydroxyanthraquinones to anthranols. The hydroxyanthraquinone without apparent solution was almost quantitatively converted into the anthranol.
1 part of the hydroxyanthraquinone was boiled for about two hours with 10 parts of stannous chloride and 50 parts of 33% hydrochloric acid.

This method was used to attempt the reduction of the following compounds.

(a) 2-methylanthraquinone.

Finely powdered 2-methylanthraquinone (4.5 g.) was suspended in the stannous chloride solution and boiled for two hours under a reflux condenser. After a few minutes boiling the solid melted.

On cooling, solidification to a plastic mass took place, and the product was removed from the solution. It was washed with concentrated hydrochloric acid and then water, when hardening took place and it was powdered, and dried at a low temperature. The product was a greenish yellow powder, m.p. 70° - 75°, yield 3.9 g. = 87%.

1 g. of the crude product was dissolved in a mixture of benzene 4 parts and light petroleum (60° - 80°) 1 part, filtered, and chromatographed on a column of neutral alumina, 30 cm. x 2 cm. The column showed a narrow green band at the top, the remainder being

pale yellow. There was no marked fluorescence. On continuing to pass the solvent, the yellow zone filtered through, and was collected in two portions of approximately 50 ml. each.

1. Yellowish white residue on evaporation, m.p. 73° - 77°, weight 0.18 g.
2. Similar appearance on evaporation, m.p. 80° - 82°, weight 0.69 g.

The two residues were mixed and crystallised from ethyl alcohol. Creamy white needles were obtained melting at 84° - 86°.

Padova (45) stated that 2-methyl-10-anthrone had m.p. 86° - 88°, while Liebermann and Mamlock (46) gave m.p. 80° - 84°. Barnett and Goodway (44) showed however that these melting points were from mixtures, and that the true melting point of 3-methyl-9-anthrone is 101°. It was thus evident that the above crystals melting at 84° - 86° were not a pure substance.

In order to attempt separation of the constituents, 1 g. of the crude reduction product was again dissolved in benzene/light petroleum mixture, and chromatographed on the same size of column as before. In this case the filtrate was collected in 10 ml. portions, each of which was evaporated to dryness, and the melting point of the residue determined. The results were:

Fraction 1. Trace only - m.p. 153° - 165°.

Fraction 2. Pale cream - m.p. 80° - 100°.
3. do. m.p. 77° - 106°.
4. do. m.p. 78° - 97°.
5. do. m.p. 77° - 97°.
6. do. m.p. 75° - 79°.
7. do. m.p. 76° - 89°.
8. do. m.p. 74° - 79°.
9. Yellowish - m.p. 74° - 85°.

Fraction 1 was evidently 2-methyl-anthraquinone.

Fractions 2 - 5 were mixed and crystallised from ethyl alcohol, m.p. 137° - 152°. On recrystallising from ethyl alcohol, the melting point rose to 165° - 172°.

Fractions 6 - 9 were mixed and crystallised from ethyl alcohol, m.p. 120° - 149°.

No separation of anthrones has thus been effected by this method.

The Dinitrophenylhydrazine Test (Allen's Method) was carried out on 0.3 g. of the crude reduction product. It was dissolved in 5 ml. warm alcohol and then heated to boiling with 5 ml. solution of 2:4-dinitrophenylhydrazine. 2-3 drops conc. hydrochloric acid was added and the solution boiled for 30 seconds. No precipitate formed on cooling. On addition of a few drops of water the solution became cloudy, but even on standing overnight no significant precipitate was formed.

(b) 2-chloroanthraquinone.

2-chloroanthraquinone (5 g.) was boiled for 2 hours with 50 g. stannous chloride A.R. and 250 ml. conc. hydrochloric acid. The substance floated at first, but after boiling for some time began to sink and by end of two hours was lying on the bottom of flask. The solid was filtered off, washed with water and dried at a low temperature. Greenish yellow powder.

Yield - 4.4 g. = 88%.

m.p. 127° - 170°.

1 g. was dissolved in 100 ml. of a mixture of benzene (3 parts) and light petroleum 60° - 80° (1 part). The solution was chromatographed on a column of neutral alumina, 30 x 2 cm. Development was continued with the same solvent, and the filtrate was collected in fractions:

1. 20 ml. - colourless. On evaporation a creamy white residue (0.2 g.) was obtained, m.p. 140° - 188°.
   This was boiled with light petroleum (60° - 80°) and filtered. The residue had m.p. 164° - 185°. The filtrate yielded crystals on cooling, which melted at 130° - 139°.
2. 60 ml. - pale yellow. On evaporation a pale yellow residue (0.41 g.) was obtained, m.p. 130° - 165°. This was boiled with light petroleum 60° - 80° and filtered. The residue melted at 139° - 180°. The

filtrate crystallised on cooling, and the crystals melted at 125° - 140°. Further recrystallisation did not raise the melting point.

3. Remainder - yellow. On evaporation gave a yellow residue melting at 158° - 172° (0.2 g.). This was rejected as the melting point was higher than that of 2-chloroanthrone (156°) reported by Barnett and Matthews (15).

(c) 1-methylanthraquinone.

The anthraquinone (5 g.) was boiled with 50 g. stannous chloride A.R. as before. During boiling the substance gradually melted. It solidified on cooling forming a brown powder, which became sticky on gently warming.

Yield - 4.5 g. = 90%.

m.p. 50° - 72°.

1 g. was dissolved in benzene and chromatographed on neutral alumina 55 x 2 cm. The solution had a yellow fluorescence. The addition of benzene was continued, and 40 ml. fractions of the filtrate were collected:

1. On evaporation a pale yellow residue was obtained weighing 0.25 g., m.p. 68° - 84°. When crystallised from dil. alcohol - m.p. 77° - 80°.
2. Yellow residue on evaporation (0.5 g.), m.p. 69° - 110°. On crystallising from dil. alcohol - m.p. 72° - 94°.
3. Yellow residue on evaporation (0.21 g.), m.p. 89° - 112°. On crystallising from

dil. alcohol - m.p. 99° - 116°.

4. Trace of residue only.

The three fractions, crystallised from dilute alcohol, were mixed and dissolved in benzene and light petroleum 60° - 80° (equal parts), and passed through a column of neutral alumina, 45 x 2 cm. Elution was continued with the same solvent. The filtrate was collected in 10 ml. fractions:

Fractions 1 and 2 yielded only traces of residue.
3 gave pale yellow residue, m.p. 92°-108°.
4 " " " " m.p. 111°-115°.
5 " " " " m.p. 105°-116°.
6 " " " " m.p. 120°-135°.
7 trace only.

Braun and Bayer (59) gave the m.p. of 1-methylanthrone as 126° - 127°, so fraction 6 was rejected.

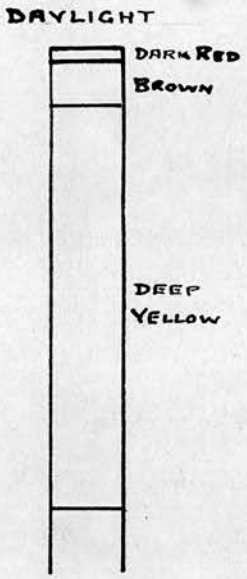
Fractions 4 and 5 were mixed and crystallised twice from light petroleum 80° - 100°, giving m.p. 119° - 123°. The amount remaining was so small that further crystallisation was impracticable.

Conversion to benzanthrones.

3 g. of the reduction product of 1-methylanthraquinone were mixed with 45 g. 82% H_2SO_4 and 3 g. of glycerin, and the mixture carefully heated to 120°. After standing overnight the mixture was poured into water. The precipitate was filtered, washed with water, and then boiled for ½ hour with 13 times (65 ml.) the quantity of 1% caustic

soda. A semi-solid residue remained. On drying and cooling 1.75 g. of a reddish brown powder was obtained.

1 g. of this powder was dissolved in benzene, filtered, and chromatographed on a column of neutral alumina, 50 x 2 cm., with the result shown. There was very little difference in ultra violet light.



On development with benzene, the yellow portion filtered through, and the filtrate was collected in 25 ml. portions and evaporated.

1. 0.03 g. m.p. 90° - 115°.
2. 0.2 g. m.p. 79° - 92°. On crystallising from alcohol 95%, m.p. 234 -36°.
3. 0.18 g. m.p. 90° - 180° when crystallised from dilute alcohol. On crystallising from 95% alcohol, m.p. 230° -235°.
4. 0.28 g. Crystallised from alcohol 95%, m.p. 233° - 245°. Mother liquor + water gave precipitate, m.p. 85° - 92°.
5. 0.12 g. Crystallised from alcohol 95%, m.p. 229° - 232°.

The fractions 2, 3, 4 and 5 were yellow solutions giving little fluorescence in ultra violet light. On addition of conc. sulphuric acid a bright yellow fluorescence resulted, which is characteristic of benzanthrones.

The high melting portions of the above, crystallised from 95% alcohol, were mixed and

recrystallised from the same solvent, and yielded reddish brown crystals, m.p. 235-7°.

Analysis of this product showed

C = 85.86%; H = 5.29%.

Calculation for $C_{18}H_{12}O$

C = 88.55%; H = 4.92%.

Sodium Hydrosulphite as Reducing Agent.

Battegay and Hueber (17) used sodium hydrosulphite to reduce anthraquinones in alkaline solution with formation of the anthrones. In order to go beyond the anthrahydroquinone stage, they stress the necessity of working with a big excess of alkali, and having a solution sufficiently dilute that the reaction products remain in solution. The method described was carried out with the following substances:

(a) 2-methylanthraquinone.

2-methylanthraquinone (10 g.) was added to one litre of water containing 100 ml. 35% caustic soda in a 2 litre flask fitted with a reflux condenser. The liquid was heated to boiling. About 40-50 g. anhydrous sodium hydrosulphite was added quickly in small portions, by quickly removing the condenser only for a sufficient time necessary to add the reducing agent. On the first addition the liquid became blood red in colour, which gradually faded to brown. The end of the reaction was noted when the addition of more sodium hydrosulphite no longer produced the blood red colour. About 12 hours boiling was

required. The liquid was quickly filtered when hot. The filtrate was acidified with hydrochloric acid, and filtered. A yellow precipitate was obtained which was dried by gentle heat overnight. The product was pale brown, weighing 6.6 g., and had m.p. 70° - 120°. On crystallising from ethyl alcohol a substance melting at 152° - 167° was obtained. This substance was not soluble in warm caustic soda solution, so could not be the original reduction product, as it was in solution of alkali before being precipitated by acid. A mixed melting point with 2-methylanthraquinone was 165° - 173°, which suggested that oxidation to the original substance had taken place. On boiling with caustic soda and sodium hydrosulphite a red colour was obtained.

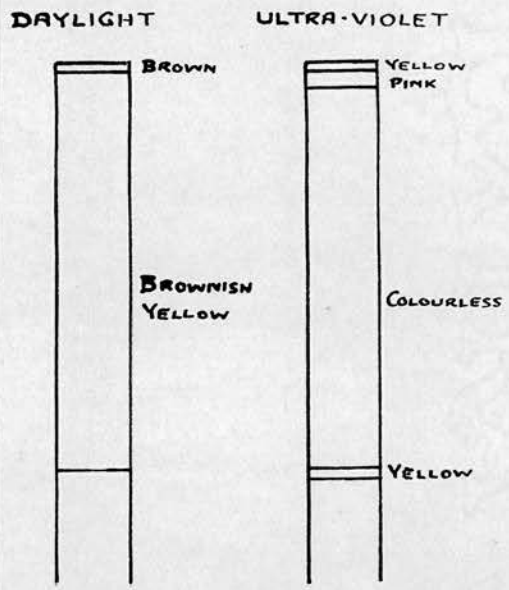
A small portion of the original precipitate which had not been dried by heating overnight, was dried very carefully and remained yellow. Its melting point was 62° - 70°.

This was soluble in warm dilute caustic soda, and on acidifying with glacial acetic acid gave a precipitate. An alcoholic solution of the latter gave a blue fluorescence in ultra violet light, indicating that an anthrone was present.

The above reduction with sodium hydrosulphite was repeated. The filtrate obtained on acidification was drained, and immediately crystallised from alcohol. The

yellow needles obtained were dried in a desiccator, m.p. 84° - 88°. On recrystallising from alcohol, m.p.= 87° - 90°.

0.4 g. recrystallised substance was dissolved in equal parts of benzene and light petroleum 60° - 80°, and chromatographed on alumina, 30 x 2 cm.



On developing with benzene 0.1 g. of a pale yellowish white residue was obtained from the filtrate on evaporation. Its melting point was 174-6° indicating the original 2-methylanthraquinone.

The solvent was changed to alcohol, and the filtrate was collected in approximately 50 ml. portions. Each portion was evaporated to dryness yielding the following amounts of residue:

1. 0.06 g. - m.p. 136° - 143°.
2. 0.03 g. - m.p. 140° - 150°.
3. 0.07 g. - m.p. 153° - 165°.
4. 0.05 g. - m.p. 98° - 130°.

All these residues gave a red colour when heated with caustic soda solution and sodium hydrosulphite.

(b) <u>anthraquinone</u>.

Anthraquinone (10 g.) was treated by the method of Battegay and Hueber. Boiling

overnight was necessary to complete the reaction. The solution was filtered while still hot. It was refiltered when cold before acidifying, then filtered and the precipitate washed with water. On drying with very gentle heat, a pale yellowish brown powder was obtained weighing 6.7 g. and melting at 210° - 250°.

It was only partially soluble in warm caustic soda, and when sodium hydrosulphite was added to this a red colour developed. (The original reduction mixture was free from red even on the addition of more sodium hydrosulphite).

The powder was only partially soluble in alcohol. 1 g. was washed with boiling alcohol and the residue dried. The melting point of the residue was 135° - 235°. On crystallising from glacial acetic acid the melting point became 198° - 235°.

The alcoholic solution was evaporated and 0.3 g. residue was obtained, m.p. 157°-180°. The melting point was unchanged when the product was recrystallised from alcohol.

Reduction of 2-methylanthraquinone by $Na_2S_2O_4$.

2-methylanthraquinone (10 g.) was treated by the method of Battegay and Hueber, with the modification that nitrogen was passed through the reaction mixture throughout the time of boiling (8 to 9 hours). The liquid was filtered while hot, and the filtrate cooled, nitrogen being passed

through it continually. It was then acidified with conc. HCl and filtered. The precipitate was washed with water, well drained and pressed between filter paper, and finally dried in a vacuum desiccator.

6.7 g. of a yellowish brown product were obtained. It was partially soluble in warm sodium hydroxide solution, suggesting the presence of the anthrone, but on addition of sodium hydrosulphite to this solution, a red colour was obtained, showing that the anthraquinone was also present.

The melting point of the crude product - 90°-105°.
On crystallising from alcohol - m.p. 139°- 55°.
Recrystallised from alcohol - m.p. 171°- 4°.
Mixed m.p. with 2-methylanthraquinone - 173°- 5°.

Reduction of 2-methylanthraquinone by $Na_2S_2O_4$ in acid solution.

2-methylanthraquinone (10 g.) was dissolved by heating in 150 ml. glacial acetic acid. 30 ml. water was added gradually with constant stirring, thus forming a suspension. 13 g. sodium hydrosulphite was added gradually over a period of about 15 minutes, the temperature being maintained at 65°. After cooling, the precipitate was filtered off.

m.p. - 172° - 5°.

Mixed m.p. with 2-methylanthraquinone - unaltered.

The above procedure was repeated, but heating was continued for 1½ hours.

The separated substance again proved to be unchanged 2-methylanthraquinone.

Reduction of 2-chloroanthraquinone by Tin, Glacial Acetic Acid and Fuming Hydrochloric Acid.

2-chloroanthraquinone (10 g.) was dissolved by heating in 75 ml. glacial acetic acid, and boiled with 10 g. granulated tin. During the boiling 20 ml. of fuming hydrochloric acid (S.G. 1.205) was added over a period of 1½ hours.

The solution was cooled and filtered. 7.5 g. of a pale yellow product was obtained, which on crystallising from alcohol gave m.p. 141° - 146°. This substance was soluble in warm caustic soda solution, and this solution gave no red colour with sodium hydrosulphite, showing that no unchanged anthraquinone remained.

Analysis: Found: C = 68.81%; H = 3.94%.

Calc. for $C_{14}H_9OCl$: C = 73.51%; H = 3.97%.

The above product was recrystallised twice from alcohol, m.p. 154-6°, (lit. m.p. of 3-chloro-9-anthrone 156°).

Analysis: Found: C = 70.91%; H = 3.96%.

This substance was completely soluble in warm caustic soda, giving a yellow solution, which on addition of $Na_2S_2O_4$ did not become red.

1 g. of the crude product was dissolved in benzene, filtered, and chromatographed on a column of alumina, 40 cm. x 2 cm. The chromatogram was yellow, the upper third being slightly darker than the remainder. When viewed under ultra violet light there was no significant change.

The column was extruded and divided into three approximately equal parts, which were eluted with warm alcohol.

Bottom zone. Evaporation of the eluate gave 0.3 g. yellow residue melting at 146-200°. It gave a red colour on warming with warm NaOH and $Na_2S_2O_4$.

Middle zone. 0.51 g. residue was obtained, m.p. 143-210°. It also gave red colour with warm NaOH solution and $Na_2S_2O_4$.

Top zone. 0.15 g. residue which again gave the red colour as above, m.p. 150-195°.

The three residues above which had been recovered from the column were mixed, washed twice with boiling alcohol, and the insoluble residue filtered off and dried. This product was insoluble in warm caustic soda solution - on addition of sodium hydrosulphite to this, a red colour was obtained.

Melting point - 195° - 212°.

Mixed m.p. with 2-chloroanthraquinone - 195°- 209°.

Analysis: Found: C = 70.94%; H = 3.48%.

Calc. for $C_{14}H_7O_2Cl$: C = 69.28%; H = 2.91%.

The above evidence therefore points to the anthrone having been oxidised on the column of alumina, with formation of the anthraquinone.

Stability of Anthrone.

During several of the reductions carried out with sodium hydrosulphite in alkaline solution, it was thought that the reduction of the anthraquinone had been successful (as was indicated by the fact that the solution in alkali and sodium hydrosulphite became free from the red colour). On working up the product, however, the presence

of the original anthraquinone was always found.

Anthrone (m.p. 153-6°) which was quite free from anthraquinone, was therefore treated in several ways in order to determine its stability.

(a) Anthrone (2 g.) was dissolved by heating with 200 ml. water and 7 g. NaOH, and 10 g. $Na_2S_2O_4$ added gradually during boiling for 2 hours. No red colour developed, thus confirming the absence of anthraquinone.

The solution was allowed to stand overnight, acidified with conc. HCl, and the precipitate filtered and washed. It was dried by heating gently in an oven - yield 1.9 g. m.p. 156-230°. The product was only partially soluble in warm caustic soda solution, and on adding $Na_2S_2O_4$ a red colour was produced, showing the presence of anthraquinone.

The bulk of the product was washed with boiling alcohol until the washings were almost colourless. The residue was dried.

m.p. - 240° - 253°.

Mixed m.p. with anthraquinone - 242° - 255°.

(b) Some of the anthrone was dissolved in warm caustic soda solution, and allowed to stand for 3 days. A small portion of the solution was then warmed with $Na_2S_2O_4$ - the solution became red.

The remainder of the solution was acidified with conc. HCl, filtered, and the precipitate washed and dried in an oven.

m.p. of crude product - 190° - 250°.

It was washed with boiling alcohol, and the

residue dried.

m.p. - 235° - 247°.

Mixed m.p. with anthraquinone - 240° - 255°.

On warming with caustic soda solution (in which it was insoluble) and adding $Na_2S_2O_4$ a red colour was produced.

(c) Anthrone was moistened with water, and dried in an oven at moderate temperature. The dried substance showed no reaction for anthraquinone.

m.p. - 153 - 6°.

Mixed m.p. with anthrone - 153 - 6°.

It is thus evident that anthrone is unstable in presence of alkali, and is readily oxidised to anthraquinone.

Reduction of 1:4-dihydroxyanthraquinone.

10 g. 1:4-dihydroxyanthraquinone

15 g. granulated tin

75 ml. glacial acetic acid

were boiled, and while boiling 20 ml. of fuming hydrochloric acid (S.G. 1.22) were gradually added over a period of 1½ hours.

The solution was then cooled, and the resulting precipitate filtered and washed with water. After drying, the crude product weighed 6 g.

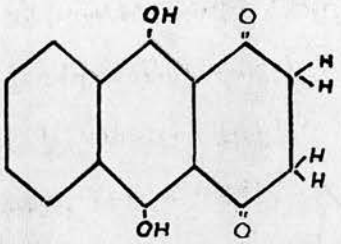
m.p. - 147° - 153°.

It was crystallised first from alcohol, and then from light petroleum (100° to 120°), giving orange yellow crystals, m.p. 156-7°, (lit. 156°).

Analysis: Found: C = 69.16%; H = 4.56%.

Calc. for 1:4-dihydroxyanthrone

$C_{14}H_{10}O_3$: C = 74.33%; H = 4.42%.



$C_{14}H_{10}O_4$ requires

C = 69.42%;

H = 4.13%.

Acetylation of reduced compound.

The above compound (4 g.) was suspended in 14 g. pyridine and 3 ml. acetyl chloride gradually added. After ¾ hour the crystals which had formed were filtered, washed with pyridine and crystallised from glacial acetic acid. m.p. 160-5°.

This crude substance was crystallised from alcohol and glacial acetic acid, and then from light petroleum (100° - 120°), giving white needles.

m.p. - 215 - 7°.

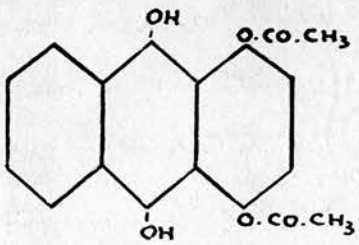
Analysis: Found: C = 65.86%; H = 4.77%.

The di-acetate of 1:4-dihydroxyanthrone $C_{18}H_{14}O_5$

requires C = 69.68%; H = 4.51%.

The mono-acetate $C_{16}H_{12}O_4$

requires C = 71.65%; H = 4.48%.



Calc. for $C_{18}H_{14}O_6$:

C = 66.26%;

H = 4.29%.

The above results agree with the views put forward by Zahn (47).

Preparation of 1:4-diacetoxyanthraquinone.

5 g. 1:4-dihydroxyanthraquinone
15 ml. acetic anhydride
5 ml. pyridine

were refluxed for 2 hours and cooled. 50 ml. alcohol were added, and the precipitate filtered. It was crystallised from glacial acetic acid, forming dull yellow crystals, m.p. 200° - 202°, (lit. 200°).

Reduction of 1:4-diacetoxyanthraquinone.

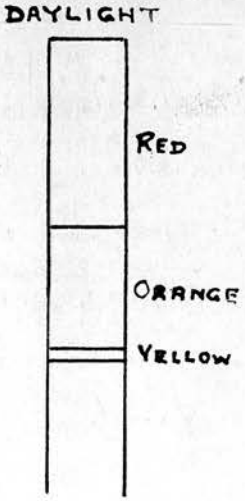
2 g. were dissolved in 14 ml. hot glacial acetic acid and precipitated by adding 14 ml. water gradually with constant stirring. 2.7 g. sodium hydrosulphite was added gradually at 65°. On cooling the resulting precipitate was filtered.

m.p. - 177° - 181°.

Mixed m.p. with original compound - 167 - 75°.

Crystallised from alcohol - m.p. 190 - 5°.

A solution in benzene was chromatographed on a column of alumina, 30 cm. x 1.2 cm., which caused a separation as shown. There was no change in ultra violet light. On developing with benzene containing 10% alcohol the orange and yellow zones filtered through. The latter gave a negligible residue. The residue on evaporation from the orange zone was crystallised from a mixture of benzene and light petroleum (60°- 80°). m.p. not below 280°.



The red zone was removed from the column and

elution was very difficult. Even boiling with alcohol only removed a small amount. This on evaporation gave a dark red substance which also did not melt below 280°.

No further work was done on this.

Reduction of 1:8-dihydroxyanthraquinone.

10 g. 1:8-dihydroxyanthraquinone
15 g. granulated tin
75 ml. glacial acetic acid

were boiled under a reflux condenser, and 20 ml. fuming HCl (S.G. 1.22) added gradually over a period of 1½ hours. On allowing to cool the crystals formed were filtered, washed with glacial acetic acid and water, and dried.

Yield - 7.4 g. m.p. - 174 - 8°.

It was crystallised from alcohol and then from light petroleum (100° - 120°) giving yellow crystals. m.p. 175 - 6°.

Analysis: Found: C = 74.05%; H = 4.86%.

Calc. for 1:8-dihydroxyanthrone

$C_{14}H_{10}O_3$: C = 74.33%; H = 4.41%.

1:8-dihydroxyanthraquinone gives a red solution in caustic soda, while the above substance gave yellow - corresponding to the anthrone.

Reduction of 1-hydroxy-3-methylanthraquinone.

1 g. 1-hydroxy-3-methylanthraquinone
1.5 g. granulated tin
7.5 ml. glacial acetic acid

were boiled under a reflux condenser and 2 ml. fuming HCl (S.G.1.22) added gradually over a period of 1½ hours. After cooling, the crystals formed were filtered, and crystallised from light petroleum.

0.5 g. yellow orange crystals, m.p. 160- 1° were obtained (lit. 158-9°).

Analysis: Found: C = 77.31%; H = 5.31%.

Calc. for 1-hydroxy-3-methylanthrone

$C_{15}H_{12}O_2$: C = 80.37%; H = 5.33%.

Solution in warm NaOH - yellow orange.

Liebermann Test - Anthrone + cooled fuming HNO_3 gave orange precipitate on adding a little water. Addition of NaOH gave red colour.

Soluble in cold benzene, crystallised from hot.

Acetylation of 1-hydroxy-3-methylanthrone.

0.2 g. anthrone

0.5 ml. acetic anhydride

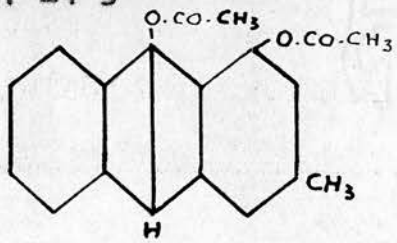
1 drop conc. H_2SO_4

were boiled for 1 minute. Water was added gradually, and the resulting precipitate filtered, washed with warm sodium bicarbonate solution. The crude substance was crystallised from alcohol and then from light petroleum (100° - 120°).

m.p. - 164- 6°.

Analysis: Found: C = 73.83%; H = 5.37%.

$C_{17}H_{14}O_3$ requires: C = 76.7%; H = 5.26%.



$C_{19}H_{16}O_4$ (the diacetate) requires

C = 74.02%; H = 5.19%.

Benzanthrone formation.

(Mayer and Fischbach (60)).

0.2 g. anthrone

3 g. 82% H_2SO_4

0.2 g. glycerin

were heated to 120° and allowed to stand overnight.

It was poured into water and filtered. It was crystallised from light petroleum (100° - 120°) and then from methyl alcohol. m.p. 132 - 5°.

Analysis: Found: C = 79.63%; H = 5.45%.

$C_{18}H_{12}O_2$ requires: C = 83.1%; H = 4.61%.

3. SYNTHESIS OF HYDROXYANTHRAQUINONES.

Preparation of 1-hydroxy-3-methylanthraquinone.

(Bentley, Gardner and Weizmann (24)).

A mixture of

30 g. phthalic anhydride

30 g. m-cresol

60 g. boric acid

was heated to 170° - 180° under a reflux air condenser for about 2 hours. The reddish brown fusion mixture was poured into about 500 ml. cold water and filtered. The residue was boiled three times with 150-200 ml. water, by which time the washings were almost free from cresol.

The residue was extracted with successive portions of sodium carbonate solution, until the extracts gave no precipitate on acidification. The mixed alkaline solution was acidified with conc. HCl, and the precipitate filtered and washed with water.

Yield of crude substance = 10 g.

On crystallising from methyl alcohol 6.3 g. of 2'-hydroxy-4'-methyl-2-benzoyl-benzoic acid were obtained. m.p. 210- 12°. (lit. 210- 11°)

2.3 g. of 2'-hydroxy-4'-methyl-2-benzoyl-benzoic acid were mixed with 4.6 g. boric acid and dissolved in conc. H_2SO_4. Fuming H_2SO_4 was added until the yellow colour of the solution changed to intense red. The mixture was poured into 500 ml. water and filtered. The residue was washed with hot water, dried, and crystallised from glacial acetic acid.

Yield of 1-hydroxy-3-methylanthraquinone - 1.3 g.

m.p. 177- 8°. (lit. 178°)

Condensation of Tetrachlorophthalic Acid and m-Cresol.

30 g. Tetrachlorophthalic Acid

30 g. m-cresol

60 g. Boric Acid

were heated for 2 hours at 200° - 225°. The fusion mixture was poured into 500 ml. water, and filtered. The residue was boiled three times with 250 ml. water, and filtered. The residue was extracted with warm sodium carbonate solution until the extract gave no precipitate with HCl. The mixed alkaline solutions were acidified with HCl, and the resulting precipitate filtered and dried.

Yield = 13.6 g. m.p. - about 225°.

The product was crystallised from glacial acetic acid. Yellowish white needles were obtained, m.p. 227- 9°, (lit. 232- 5°).

Analysis: Found: Cl = 34.70%.

Calc. for $C_{15}H_8O_4Cl_4$: Cl = 35.98%.

Acetylation.

The above compound (3 g.) was boiled for 1 minute with 5 ml. acetic anhydride containing 5 drops conc. H_2SO_4. On pouring into water a precipitate was obtained which on crystallising from glacial acetic acid gave white needles - m.p. 163- 5°. Yield - 2 g.

Analysis: Found: Cl = 28.83%.

Acetyl derivative

$C_{17}H_{10}O_5Cl_4$ requires Cl = 32.53%.

Ring-closure.

Ring-closure of the benzoylbenzoic acid by the method of Bentley, Gardner and Weizmann was not successful. On pouring the red sulphuric acid mixture into water no precipitate occurred.

Ring-closure of benzoyl-o-benzoic acid.

Benzoyl-o-benzoic acid (2 g.) was treated by the method of Waldmann (29) except that o-dichlorbenzol was used instead of trichlorbenzol.
The product when filtered yielded 1.25 g. crystals.

m.p. - 150° - 230°.

Crystallised from glacial acetic acid - m.p. 283-5°.
Mixed m.p. with anthraquinone - 282 - 5°.

Ring-closure of 2'-hydroxy-4'-methyl-3:4:5:6-tetrachlorobenzoylbenzoic acid.

Hydroxymethyltetrachlorobenzoylbenzoic acid (2 g.) was treated by the above method. On cooling the mixture no solid separated. Ether was added, and the solution extracted with sodium carbonate solution to remove excess benzoyl chloride and any unchanged acid. The ethereal layer was separated and the ether allowed to evaporate. The solid residue was dissolved in benzene, filtered, and an equal quantity of light petroleum (80° - 100°) added. On standing overnight crystals formed.

m.p. - 204 - 6°.

Analysis: Found: Cl = 22.4%.

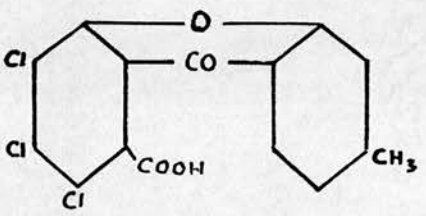
$C_{15}H_6O_3Cl_4$ requires Cl = 37.77%.

$C_{15}H_8O_3Cl_2$ requires Cl = 23.1%.

Action of alkali on hydroxymethyltetrachloro-benzoylbenzoic acid.

When the benzoylbenzoic acid was dissolved in sodium hydroxide solution, it was noted that solution took place rapidly, but immediately afterwards a precipitate began to form. After standing overnight, the precipitate was filtered, boiled with conc. HCl, washed with water, and crystallised from glacial acetic acid. White needles were formed, m.p. 268 - 70° with decomposition.

Analysis: Found: Cl = 28.43%.



Calc. for $C_{15}H_7O_4Cl_3$:
Cl = 29.8%.
m.p. (lit.) 254°.

Condensation of 3-nitro-phthalic anhydride with m-cresol.

3-nitro-phthalic anhydride (30 g.) was treated by the method of Bentley, Gardner and Weizmann as described previously. After removal of the excess m-cresol by boiling with water, extraction with hot sodium carbonate solution was carried out.

On acidification of the alkaline solution a brown resinous mass was obtained which could not be obtained crystalline.

Preparation of 3-chlorophthalic acid.

3-aminophthalic acid was first prepared by the method of Kenner and Matthews (61) using the following quantities:

Zinc dust	15 g.
3-nitrophthalic acid	25 g.
20% acetic acid	300 g.
sodium acetate cryst.	150 g.

The product of this reaction was diazotised in the normal manner.

The diazotisation solution was then added slowly, with constant shaking, to an ice cold solution of cuprous chloride, and the mixture allowed to stand overnight. No crystallisation took place.

The solution was concentrated to about 150 ml. and on cooling yellow crystals were formed. These were dissolved in the minimum of boiling water, filtered, and allowed to crystallise.

Yield - 3.5 g. m.p. - 175 - 85°.

On recrystallising from water

m.p. = 182 - 4° with decomposition (lit. 186°).

<u>Condensation of 3-chlorophthalic acid with m-cresol.</u>

(i) By method of Bentley, Gardner and Weizmann (24).

3-chlorophthalic acid	3 g.
m-cresol	3 g.
boric acid	6 g.

were heated at 180 - 200° for 2 hours. The fusion mixture was poured into cold water and filtered. The residue was boiled with water, when it melted, making filtration impossible. The cooled aqueous mixture was extracted with chloroform and the chloroform distilled off.

The residue was partially soluble in alcohol.

(a) The insoluble portion was crystallised from glacial acetic acid giving yellowish white needles m.p. 253 - 5°.

Analysis: Found: Cl = 8.93%.

Calc. for $C_{15}H_{11}O_4Cl$: Cl = 12.22%.

(b) The alcoholic solution was evaporated to dryness. The residue was dissolved in sodium carbonate solution, filtered, and acidified with hydrochloric acid. The resulting precipitate was crystallised from glacial acetic acid and recrystallised from diluted acetic acid. Yellowish white needles were obtained, m.p. 220 - 221°.

Yield = 0.1 g.

Analysis: Found: Chlorine = 12.17%.

Calc. for $C_{15}H_{11}O_4Cl$ = 12.22%.

(ii) By method of Ullmann and Schmidt (23).

3-chlorophthalic acid	3 g.
m-cresol	3 g.
tetrachloroethane	15 ml.
aluminium chloride	6 g.

were heated for 2½ hours as described in above method. The crude substance obtained was crystallised from diluted acetic acid.

m.p. = 215 - 216.5°. Yield = 0.1 g.

Analysis: Found: Chlorine = 12.39%.

Calc. for $C_{15}H_{11}O_4Cl$ = 12.22%.

Mixed m.p. with previous compound = 217 - 8°.

<u>Condensation of 3:6-dichlorophthalic acid with m-cresol</u>.

(i) By method of Ullmann and Schmidt (23).

3:6-dichlorophthalic anhydride	10 g.
m-cresol	10 g.
tetrachloroethane	50 ml.
aluminium chloride	25 g.

were heated together as before for 2½ - 3 hours.

A semi-solid mass was obtained which formed a viscous yellowish brown lower layer on mixing with water. This was washed three times with about 500 ml. water, and then extracted with hot dilute ammonia solution. On continued extraction with hot ammonia solution, the residue gradually solidified. Extraction was continued until the extract was almost colourless.

The combined extracts were concentrated to about $^2/_3$ volume, filtered, and then acidified with hydrochloric acid. A resinous precipitate was obtained which solidified on cooling. This was crystallised from glacial acetic acid.

Yield = 8.2 g. m.p. 187 - 192°.

It was recrystallised from benzene, giving yellowish white needles, m.p. 190 - 191°.

Analysis: Found: Chlorine = 21.83%.

Calc. for $C_{15}H_{10}O_4Cl_2$ = 21.6%.

(ii) By method of Bentley, Gardner and Weizmann (24).

3:6-dichlorophthalic anhydride	10 g.
m-cresol	10 g.
boric acid	20 g.

were heated for 2 hours at 180 - 200° and worked up as before. The precipitate obtained was crystallised from glacial acetic acid - yield 1.7g., m.p. 185 - 188°.

It was recrystallised from benzene m.p. 190-191°.

Mixed m.p. with product of method (i) = 190-191°.

<u>Ring-closure of 2'-hydroxy-4'-methyl-3:6-dichlorobenzoylbenzoic acid</u>.

(a) 0.5 g. of the above benzoylbenzoic acid was heated in test tube with 2.5 ml. conc. H_2SO_4 on

a water bath for 1 hour.

It was then poured into 25 ml. iced water.

No precipitation occurred.

(b) 0.5 g. was heated in a test tube with 10 ml. sulphuric acid (3 vol. conc. H_2SO_4 + 1 vol. water). The yellow solution gradually turned reddish, and at about 160° crystals began to form. The mixture was cooled to room temperature and diluted with water. The precipitate was filtered and crystallised from benzene, and recrystallised from benzene and light petroleum.

m.p. 191.5 - 193°. Yield small.

Mixed m.p. with original = 155° - 180°.

(c) 0.5 g. + 1 g. boric acid were dissolved in conc. H_2SO_4 and fuming H_2SO_4 added. The mixture was poured into 100 ml. water and filtered.

Yield - 0.25 g. m.p. 184 - 187°.

Mixed m.p. with original = 187 - 189°.

(d) 0.5 g. was added to 10 ml. conc. H_2SO_4 previously heated to 160 - 170°, and kept at that temperature for 5 minutes. It was cooled and poured into 100 ml. water. When filtered, the yield was found to be negligible.

(e) 0.5 g. was added to 10 ml. conc. H_2SO_4 at 160° and maintained at that temperature for 15 minutes. The solution was cooled and a small amount of water (1 - 1.5 ml.) was added gradually without allowing the temperature to rise. The mixture was filtered and the residue washed with water.

Yield - 0.1 g. m.p. 191 - 193°.

On crystallising from benzene and light

petroleum almost white needles were obtained, m.p. 193 - 194°.

The mixed m.p. with the product from method (b) was unchanged.

Mixed m.p. with the original substance - 155-185°.

Analysis: Found: C = 44.25%; H = 0.99%; Cl=30.5%.

$C_{15}H_8O_3Cl_2$ requires: C = 57.75%; H = 2.62%; Cl=22.9%.

Calc. for $C_8H_2O_3Cl_2$: C = 44.4%; H = 0.9%; Cl=32.4%.

The melting point of 3:6-dichlorophthalic anhydride ($C_8H_2O_3Cl_2$) is given in the literature as 194 - 5°.

Condensation of 3:6-dichlorophthalic anhydride with p-chloro-m-cresol.

3:6-dichlorophthalic anhydride	10 g.
p-chloro-m-cresol	10 g.
tetrachloroethane	50 ml.
aluminium chloride	25 g.

were treated by the method of Ullmann and Schmidt (23).

Yield of crude substance = 13.7 g.

It was crystallised from glacial acetic acid, and then from benzene and light petroleum, yielding white needles, m.p. 189 -190.5°.

Analysis: Found: C = 49.87%; H= 2.25%; Cl= 27.32%.

Calc. for $C_{15}H_9O_4Cl_3$: C = 50.07%; H = 2.50%; Cl= 29.63%.

Ring-closure of 2'-hydroxy-4'-methyl-5'-chloro-3:6-dichlorobenzoylbenzoic acid.

(a) 0.5 g. was treated by method of Eder and Widmer (62).

This yielded 0.15 g. crude product, which gave yellowish needles on crystallising from benzene and alcohol. m.p. - above 285°.

This substance was not investigated further as it gave no reactions for anthraquinones.

(b) 0.5 g. was treated by method of Eder and Widmer (49). The crude residue after filtration was boiled with water containing sodium carbonate, filtered, washed with water, and dried.

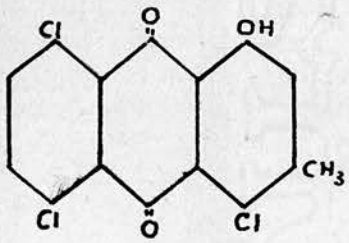
Yield = 0.37 g.

It was crystallised from benzene and then recrystallised from benzene and light petroleum giving orange needles.

m.p. = 201 - 201.5°.

This substance gave a red colour when heated with caustic soda solution and sodium hydrosulphite. It gave no colour with alkali alone.

Analysis: Found: C = 53.49%; H = 2.54%; Cl = 30.47%.



Calc. for $C_{15}H_7O_3Cl_3$:
C = 52.71%; H = 2.05%;
Cl = 31.18%.

Acetylation with acetic anhydride and crystallisation from glacial acetic acid gave pale yellow crystals, m.p. 198 - 200°. Recrystallisation from alcohol gave yellow needles, m.p. 201 - 202°.

Mixed m.p. with original substance = 165 - 180°.

Analysis: Found: Cl = 28.04%.

$C_{17}H_9O_4Cl_3$ requires: 27.77% Cl.

(c) (Personal communication, W.Smith, Scottish Dyestuffs Ltd., Grangemouth).

0.5 g. were added to 5 ml. conc. H_2SO_4 at 160° and maintained at that temperature for 2 hours. The solution was cooled to 80°, and

sufficient water added gradually with constant stirring to dilute the acid to 85%, keeping the temperature below 100°. When all the water was added the mixture was heated to 120° for 15 minutes, and then allowed to cool, without stirring, to 20°. The precipitate was filtered on a sintered glass funnel, and washed with a small amount of 86% H_2SO_4. It was then boiled with water made alkaline with sodium carbonate, filtered, washed acid free, and dried.

Yield - 0.05 g. m.p. 178 - 85°.

In view of the poor yield obtained by this method with the above compound, it was of interest to apply the same method to 3:4:5:6-tetrachloro-2-benzoylbenzoic acid. 1 g. of this compound gave a yield of 0.4 g. m.p. 190 - 192°. (W. Smith 191 - 2°.)

The anthraquinone "vat" test was positive, but no red colour was produced with alkali alone.

Ring-closure of 2'-hydroxy-4'-methyl-3:6-dichloro-benzoylbenzoic acid.

By method of Eder and Widmer (49).

This method, which had proved successful with the compound having an additional chlorine in the 5' position, when applied to the above compound did not give successful results. When the hot reaction mixture was poured into ice it was almost completely soluble, indicating that sulphonation had probably taken place.

Condensation of 3:6-dichlorophthalic anhydride with m-cresol.

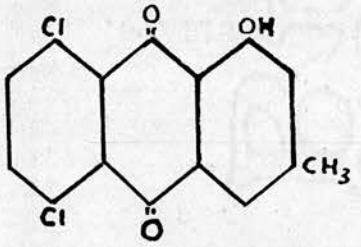
By method of Waldmann and Sellner (21).

3:6-dichlorophthalic anhydride	6.6 g.
m-cresol	4.4 g.
sodium chloride	12 g.
aluminium chloride	60 g.

were treated by the above method. A dark green residue was obtained which was boiled with sodium carbonate solution. The insoluble portion was partially soluble in benzene. The benzene solution was concentrated, and 0.8 g. yellow crystals obtained. On recrystallising from benzene and light petroleum, m.p. = 233 - 235°.

This substance gave a red colour with warm caustic soda solution and sodium hydrosulphite, but not with alkali alone.

Analysis: Found: C = 58.0%; H = 2.80%; Cl = 25.82%.



Calc. for $C_{15}H_8O_3Cl_2$:
C = 58.64%; H = 2.61%;
Cl = 23.13%.

Reduction of 2'-hydroxy-4'-methyl-5'-chloro-3:6-dichlorobenzoylbenzoic acid.

(a) by method of Ullmann (30).

1 g. of the above compound was boiled for 1 hour under a reflux condenser with

Zinc strips	2 g.
Glacial Acetic Acid	8 ml.
Water	2 g.

The liquid was decanted - no precipitate formed

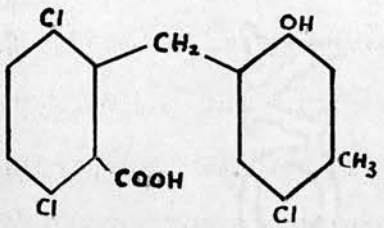
on cooling. On adding water an oily precipitate formed which hardened on standing, forming an almost white powder. Yield - 0.8 g.

Crystallised from light petroleum 100 - 120°
- m.p. 165 - 70°.

Recrystallised - m.p. 165°-168°.

Recrystallised from glacial acetic acid
- m.p. 185°-188°.

Analysis: Found: Chlorine = 26.61%.



Calc. for $C_{15}H_{11}O_3Cl_3$:
Chlorine = 30.83%.

This compound appears to be the unchanged keto-acid. The low chlorine content agrees with that obtained in the analysis of the original compound (p. 100).

(b) by method of Scholl et alt. (31).

2 g. were dissolved in 30 ml. 2N caustic soda solution, and boiled for 15 hours with 2.4 g. zinc dust. The mixture was filtered hot, and the residue washed with hot water. The filtrate and washings were acidified with hydrochloric acid, and the precipitate separated.

Yield - almost 2 g. m.p. 120 - 230°.

The crude product was almost completely soluble in hot benzene. The solution was filtered, and the filtrate cooled. Filtration yielded only a small quantity of a pale brown non crystalline product, m.p. 214 - 235° which was not inventigated further.

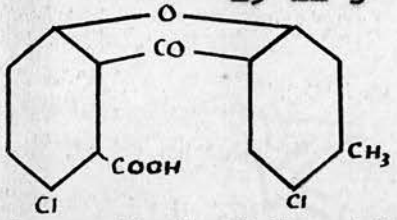
The filtrate was diluted with an equal

quantity of light petroleum (60 - 80°), and the resulting precipitate was crystallised from a mixture of benzene and methyl alcohol.

Reddish brown crystals were obtained. These gradually darkened over 200° and melted at 285°.

Analysis: Found: Chlorine = 20.09%.

Calc. for $C_{15}H_{11}O_3Cl_3$: Cl = 30.83%.



Calc. for $C_{15}H_8O_4Cl_2$: Cl = 21.98%.

(c) by method of Barnett and Wiltshire (32).

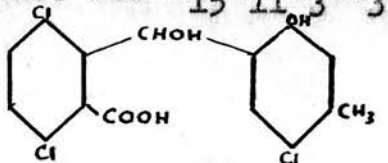
2 g. of the benzoylbenzoic acid was heated on a water bath for 22 hours with

Ammonia Solution (d.0.880)	12 ml.
Water	6 ml.
Zinc Dust	5 g.
Copper Sulphate Solution	5 drops.

The reaction mixture was filtered while hot, and the residue in the flask washed with about 10 ml. boiling water. The mixed filtrate was acidified with hydrochloric acid. An oily precipitate was formed which solidified when cooled in ice, but softened on handling. Crude yield = 0.25 g. It was dissolved in glacial acetic acid and filtered through charcoal. The precipitate formed by dilution with water had m.p. 179 -181°, but was non crystalline.

Analysis: Found: Chlorine = 28.58%.

Calc. for $C_{15}H_{11}O_3Cl_3$ Cl = 30.83%.



Calc. for $C_{15}H_{11}O_4Cl_3$: Cl = 29.38%.

The same method was repeated, but in this case the quantities of ammonia and water were doubled. The precipitate obtained on acidification was boiled with dilute hydrochloric acid to remove any traces of zinc. An easily powdered product was obtained, which weighed 0.5 g.

This was precipitated from diluted acetic acid as above, and then crystallised from benzene.

m.p. - 191 - 192°.

Mixed m.p. with original substance - 165 - 182°.

Mixed m.p. with first product - 185 - 188°.

Analysis: Found: Chlorine = 29.65%.

The zinc residues were extracted with ethyl alcohol, and the latter evaporated. About 1 g. of a brown product was obtained. This was boiled with dilute hydrochloric acid, and dried. It was dissolved in hot benzene and filtered through charcoal. On cooling, a small precipitate was obtained, m.p. 225 - 250°. The filtrate was diluted with twice its volume of light petroleum (60 - 80°). The resulting precipitate was non crystalline, and was very soluble in methyl alcohol, ethyl alcohol, and glacial acetic acid, but less so in benzene. It was insoluble in light petroleum. Attempts at crystallisation were not successful.

m.p. 205 - 235°.

Analysis: Found: Chlorine = 20.05%.

0.3 g. of the product obtained from the zinc residues was dissolved in 10 ml. 10% caustic soda solution, and boiled for 1 hour. After cooling the solution was acidified with acetic acid, and the precipitate filtered.
m.p. 265 - 285°.

On crystallising from diluted methyl alcohol, a product was obtained which softened between 285° and 290°, but did not melt.

Mixed melting point with the product from the Zn + NaOH reduction was 230 - 240°.

As the doubled amount of ammonia and water used in the method of Barnett and Wiltshire had given an improved yield, another repetition was carried out on 1 g. of the benzoylbenzoic acid, using sufficient ammonia (72 ml.) and water (36 ml.) to ensure complete solution of the compound before adding the zinc. This was successful in again doubling the yield, 0.5 g. of crude product being obtained. Crystallisation from benzene gave the same compound as before. m.p. 189 - 191°.
Mixed m.p. with previous compound - 189 - 191°.

Ring-closure of 2'-hydroxy-4'-methyl-5'-chloro-3:6-dichlorobenzylbenzoic acid.

This was carried out by the method of Barnett and Wiltshire (32) except that the volume of concentrated sulphuric acid was increased to bring the compound into solution. 0.3 g. required about 5 ml. acid. After standing for 1 hour at room temperature, the solution was poured into ice, and

filtered.

The product was dissolved in hot benzene and allowed to stand to crystallise. m.p. 180 - 185°.

The "vat" test was negative.

Purification of 1:4:5:8-tetrachloroanthraquinone.

50 g. of mixed samples of chlorine content varying from 40.72% to 42.58% (theory 41.04%) were heated with 300 ml. conc. H_2SO_4 at 50° for 2 hours. The mixture was filtered when cool, and washed thoroughly with water until free from acid. The filtrate would probably contain the low chlorinated compounds. The washed and dried product was crystallised from nitrobenzene to remove any highly chlorinated material.

Analysis: Found: Chlorine = 41.07%.

Calc. for $C_{14}H_4O_2Cl_4$: Chlorine = 41.04%.

Replacement of chlorine by hydroxyl in 1-hydroxy-3-methyl-4:5:8-trichloroanthraquinone.

(a) by method of Ullmann and Schmidt (33).

1-hydroxy-3-methyl-4:5:8-trichloro-anthraquinone	0.5 g.
boric acid	2.4 g.
conc. H_2SO_4	10 ml.

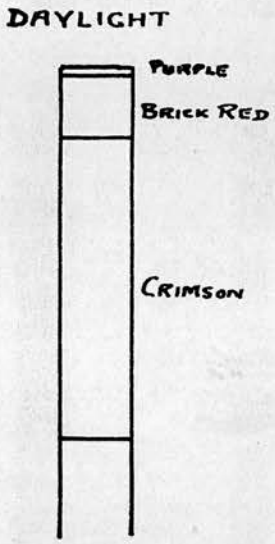
were treated by the above method.

The crude red product weighed 0.37 g. m.p.185 - 210° approx.

Crystallisation of the crude product was not successful. Hot concentrated solutions in glacial acetic acid, ethyl alcohol, and benzene + light petroleum, when cooled gave non crystalline precipitates.

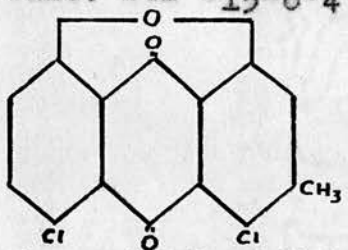
0.2 g. was dissolved in benzene and filtered.

The solution was chromatographed on magnesium carbonate (18 cm. x 2 cm.) and developed with benzene. The crimson zone filtered through, and on evaporation gave a yellowish residue weighing 0.15 g. This was crystallised from benzene. m.p. 212 - 214°.



The mixed m.p. with the original compound was 190 - 195°.
On recrystallising from benzene, m.p. 215 - 218°.

Analysis: Found: Chlorine = 24.93%.
Calc. for $C_{15}H_8O_4Cl_2$: Chlorine = 21.73%.
$C_{15}H_6O_3Cl_2$ requires Cl = 23.28%.



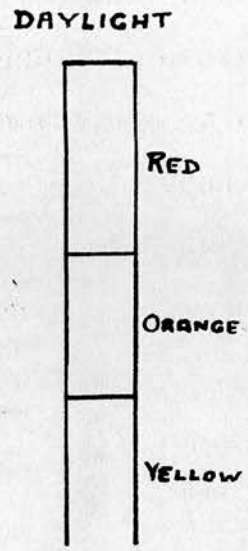
(b) by method of Keimatsu, Hirano and Yoshimi (39).

1-hydroxy-3-methyl-4:5:8-trichloro-anthraquinone	1.2 g.
Sodium	1 g.
Methyl Alcohol	20 ml.

were heated in a sealed tube for 6 hours at 160 - 180°. When cool, the reaction mixture was poured into water, and filtered. The small insoluble portion was rejected.

The filtrate was acidified with hydrochloric acid, and the resulting precipitate when filtered and dried, weighed 0.6 g.

Crystallisation from the usual solvents not being successful, it was dissolved in a mixture



of benzene and methyl alcohol, and chromatographed on a column of alumina (35 cm. x 2 cm.) with the result shown. The pale yellow zone filtered through, and on evaporation of the filtrate, the residue was negligible.

The two main zones were extruded and separated mechanically. Each was extracted in a soxhlet apparatus with a mixture of equal volumes of glacial acetic acid and ethyl alcohol. On concentration of the extract, alumina was precipitated. In order to remove the inorganic matter, concentrated hydrochloric acid was added to each extract, and then water. The mixture was shaken with chloroform in a separator, and the chloroform layers evaporated.

(1) Top zone. 0.3 g. residue.

Crystallised from diluted alcohol - m.p. 120-160°.

Crystallised twice from mixture of glacial acetic acid and chloroform gave brown needles.

m.p. 285°.

Tested for presence of chlorine - positive.

Analysis: Found: C = 14.45%; H = 4.39%; Cl = 22.31%.

Residue = 68.11%.

(2) Lower zone. 0.3 g. residue.

Crystallised from diluted acetic acid -

m.p. 145 - 210°.

Crystallised from mixture of glacial acetic acid and chloroform - m.p. 180 - 210°.

Tested for presence of chlorine - positive. Further work was not carried out on this, as it was obvious that the chlorine had not been replaced by methoxyl.

Replacement of Chlorine by Hydroxyl in 1:4:5:8-tetrachloroanthraquinone.

(a) by method of Ullmann and Schmidt (33).

1:4:5:8-tetrachloroanthraquinone	2.5 g.
boric acid	12 g.
conc. H_2SO_4	50 ml.

The above quantities were treated by this method, but only a very small amount of the product was soluble in caustic soda solution, giving a purple solution. The amount in solution was so small that on acidification with acetic acid the colour changed to yellow but no precipitate was formed.

(b) by method of Fischer and Sapper (38).

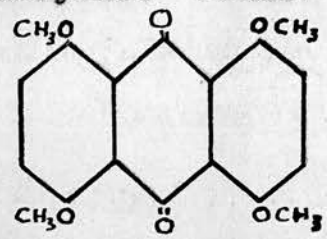
1:4:5:8-tetrachloroanthraquinone	1 g.
40% potassium hydroxide in methyl alcohol	20 ml.

were heated for 5 hours at 100° under pressure. The product, after filtering, was crystallised from glacial acetic acid, yielding pale yellow needles which did not melt below 300°. The substance gave a reaction for chlorine, and probably consisted of the original substance.

The acetic acid filtrate was concentrated, and a small quantity of yellow needles was obtained. A solution in benzene and light petroleum was allowed to evaporate slowly, giving yellow needles, m.p. 142 - 5°. Chlorine was

absent.

Analysis: Found: C = 51.71%; H = 2.61%.



Calc. for $C_{18}H_{16}O_6$:
C = 65.8%; H = 4.9%.

The second half of the method was not proceeded with.

(c) by method of Frey (36).

1:4:5:8-tetrachloroanthraquinone	2 g.
copper bronze	0.1 g.
water	30 g.
slaked lime	8 g.

were heated together in a sealed tube at 250° for 20 hours. A purple mixture resulted, which was boiled with dil. HCl. On filtering and drying 1. 5 g. of a brown product was obtained.

m.p. over 290°.

Part of this was purified by sublimation at 210 - 230°. Dark reddish brown shining needles were obtained, which gave a purple solution in caustic soda, crimson in conc. H_2SO_4 with orange-red fluorescence, and the same fluorescence in methyl-naphthalene.

Analysis: Found: C = 66.51%; H = 3.15%.
Calc. for $C_{14}H_8O_6$: C = 61.8%; H = 2.9%.
Calc. for $C_{14}H_8O_5$: C = 65.62%; H = 3.12%.

Another portion of the crude product was crystallised from benzene, sublimed, and recrystallised from benzene.

Reddish orange needles were obtained,

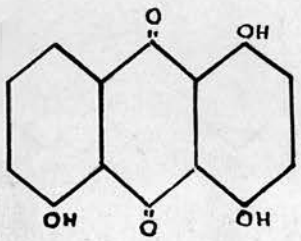
m.p. 263 - 5°.

Solution in caustic soda - violet.

Solution in conc. H_2SO_4 - magenta.

Solution in benzene - orange fluorescence.

Analysis: Found: C = 65.64%; H = 3.28%.

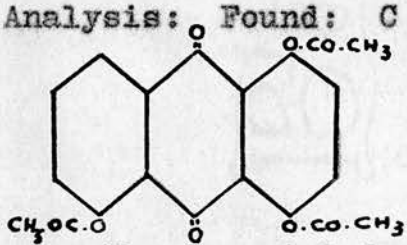


Calc. for $C_{14}H_8O_5$:
C = 65.62%; H = 3.12%.

The above method was repeated with the same quantities, but heating for only 15 hours at 240°. The crude product was extracted with boiling caustic soda solution, filtered, and the filtrate was acidified with dil. HCl giving a yield of 0.85 g.

This was boiled with acetic anhydride containing a trace of conc. H_2SO_4. The acetyl derivative, after crystallising three times from methyl alcohol, formed yellow needles, m.p. 216 - 7°.

Analysis: Found: C = 62.73%; H = 3.88%.



$C_{20}H_{14}O_8$ requires:
C = 62.81%; H = 3.66%.

The 4-acetyl derivative of 1:4:5-trihydroxy-anthraquinone is the only acetyl derivative of this substance reported in the literature.

An attempt was now made to find out if less severe conditions would allow this reaction to produce the tetrahydroxyanthraquinone.

(i) boiling under a reflux condenser for 20 hours.

No visible change took place.

(ii) heating under pressure at 110° for 20 hours.

No visible change took place.

(iii) heating under pressure at 150° for 28 hours.

The reaction mixture assumed a pale violet colour. On acidifying and filtering as formerly, 1.6 g. of a pale brown product was obtained. Only a trace of this was soluble in caustic soda solution, showing that no appreciable quantity of hydroxy-compound has been formed.

(iv) heating at 190° for 16 hours <u>without copper</u>.

A slightly deeper colour was produced than in the previous experiment. Again 1.6 g. of crude product was obtained, and from this 0.1 g. of alkali soluble material was obtained. This gave a violet colour with conc. H_2SO_4. The alkali insoluble material, on crystallising from benzene was found to be the original 1:4:5:8-tetrachloroanthraquinone.

(v) heating at 200° - 215° for 20 hours <u>without copper</u>.

The reaction mixture became purple. On working up as before 0.4 g. alkali soluble product was obtained. This was purified by sublimation at 200° - 210°, and crystallisation from benzene.

Analysis: Found: C = 66.21%; H = 3.08%.

Calc. for $C_{14}H_8O_5$: C = 65.62%; H = 3.12%.

Replacement of Chlorine by Hydroxyl in 1-hydroxy-3-methyl-4:5:8-trichloroanthraquinone.

1-hydroxy-3-methyl-4:5:8-trichloroanthraquinone	1 g.
copper bronze	0.05 g.
water	15 g.
slaked lime	4 g.

were heated at 250° for 20 hours as formerly. A purple product was obtained, which on boiling with dil. HCl, gave 0.8 g. of a dark brown substance. It was purified by sublimation at about 140°. Reddish brown needles were obtained, m.p. 155 - 7°.

Analysis: Found: C = 67.57%; H = 3.86%.

Calc. for $C_{15}H_{10}O_6$: C = 62.93%; H = 3.50%.

Calc. for $C_{15}H_{10}O_5$: C = 66.66%; H = 3.70%.

The above reaction was repeated, but heating was restricted to 18 hours at 200 - 210°. 0.75 g. product was obtained, which, on extracting with caustic soda solution, yielded 0.6 g. of alkali soluble material.

Part of this was purified by sublimation.

Red needles were obtained, m.p. 155 - 8°.

Analysis: Found: C = 68.31%; H = 3.83%.

Calc. for $C_{15}H_{10}O_5$: C = 66.66%; H = 3.70%.

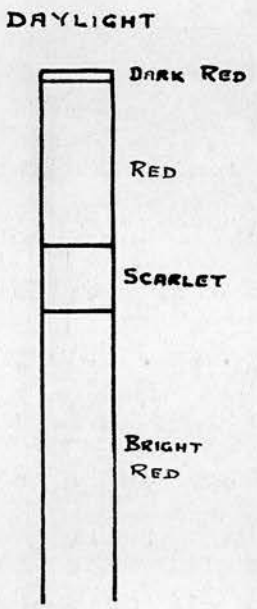
A further portion was crystallised from benzene. Dark red needles were obtained, m.p. 195 - 200°.

Analysis: Found: C = 63.53%; H = 3.38%.

Calc. for $C_{15}H_{10}O_6$: C = 62.93%; H = 3.50%.

A further portion was acetylated in the usual way. After pouring the acetic anhydride solution into water, filtration was very difficult. To

overcome this the liquid was extracted with benzene in a separator. The benzene solution was passed through a column of alumina, 25 cm. x 12 mm.



Development was carried out with benzene containing 25% alcohol. Development started fairly rapidly, but after standing overnight, even hot alcohol was not a strong enough eluant. This suggested that hydrolysis of the acetate had occurred.

No more of the substance was available for further investigation.

A further repetition of this reaction was carried out. The crude product was found to be not very soluble in caustic soda solution, so the residue (after a preliminary extraction with caustic soda) was dissolved in acetone, filtered, and the filtrate evaporated to dryness. The reddish black residue was sublimed under vacuum at about 170°. Brick red needles were obtained which were recrystallised from benzene, m.p. 172° - 174°.

Analysis: Found: C = 67.0%; H = 3.97%.

Calc. for $C_{15}H_{10}O_5$: C = 66.66%; H = 3.70%.

Replacement of Chlorine in the α-position.

Investigation of Frey's method for replacing chlorine by hydroxyl was continued by carrying out the reaction on various chloroanthraquinones with the chlorine in the α-position.

(a) 1-chloroanthraquinone.

(i) 1-chloroanthraquinone (2 g.) was treated by Frey's method, but heating was restricted to 16 hours at 190°. 1.75 g. of crude product was obtained, which was only slightly soluble in caustic soda, giving a yellow solution. The alkali insoluble portion on crystallising from alcohol gave yellow needles, m.p. 159-162°. Mixed melting point with 1-chloroanthraquinone was not depressed.

(ii) The reaction was repeated, but heating was increased to 20 hours at 200 - 215°.

0.5 g. of a yellow alkali soluble product was obtained. On crystallising from benzene orange yellow needles were obtained, m.p. 196 - 7°.

Analysis: Found: C = 74.91%; H = 3.74%.

Calc. for $C_{14}H_8O_3$: C = 75.00%; H = 3.57%.

The literature describes 1-hydroxyanthraquinone as being orange-red, and having m.p. 193°.

(b) 1:5-dichloroanthraquinone.

1:5-dichloroanthraquinone (2 g.) was treated by Frey's method, with heating for 22 hours at 200° - 215°. The product was almost completely soluble in caustic soda solution (red colour). Acidification of the alkaline solution gave 1.7 g. of a yellow product, which on crystallisation from glacial acetic acid gave yellow needles.

m.p. 275 - 8°.

Mixed melting point with 1:5-dihydroxyanthraquinone showed no depression.

(c) 1:4-dichloroanthraquinone.

1:4-dichloroanthraquinone was prepared by the method described by, and using the quantities stated by, Phillips (63).

Yield - 2 g. m.p. 187 - 8°. (lit.187.5°).

1:4-dichloroanthraquinone (1 g.) was treated by Frey's method, heating for 22 hours at 200 - 215°. 0.4 g. of alkali soluble product was obtained, which on crystallising from glacial acetic acid, gave red needles, m.p. 196 - 198°. Mixed melting point with 1:4-dihydroxyanthraquinone showed no depression.

(d) 1:8-dichloroanthraquinone.

Anthraquinone-1:8-disulphonic acid (10 g.) was chlorinated by the method described by Goldberg (56). 5.5 g. of pale yellow needles were obtained.

m.p. 204° - 205°. (lit. 202° - 3°).

Analysis: Found: Chlorine = 24.87%.

Calc. for $C_{14}H_6O_2Cl_2$: Chlorine = 25.63%.

1:8-dichloroanthraquinone (2 g.) was heated with calcium hydroxide, copper and water for 20 hours at 210° - 215° as formerly. After cooling, acidification with dilute hydrochloric acid and filtering, an orange product was obtained, which when dry weighed 1.9 g.

m.p. 191° - 195°.

Crystallisation from glacial acetic acid gave orange needles, m.p. 193° - 194°.

Mixed melting point with 1:8-dihydroxyanthraquinone was unchanged.

Attempted Preparation of 1:4:5-trichloroanthraquinone.

By method of Goldberg (54).

(a) The method described by Goldberg was carried out using red mercuric oxide as catalyst, and using half the stated quantities.

6 g. of the presumed 1-chloroanthraquinone-4:5-disulphonate was obtained.

3 g. of this was chlorinated as described, yielding 2 g. of product, m.p. 190 - 230°. On crystallising twice from glacial acetic acid, yellow needles were obtained, m.p. 248 - 50°.

Melting point after mixing with 1:5-dichloroanthraquinone was unchanged.

Analysis: Found: Cl = 23.36%.

Calc. for $C_{14}H_5O_2Cl_3$: Cl = 34.19%.

Calc. for $C_{14}H_6O_2Cl_2$: Cl = 25.63%.

(b) The same method was repeated, but the mother liquor remaining after two crystallisations was precipitated by addition of alcohol, as it was hoped that it would contain some disulphonate, if any were present.

About 20 g. of a yellow solid were obtained, 10 g. of which were chlorinated as before.

2 g. of product were obtained - m.p. 160 - 170°.

1 g. of this substance was dissolved in benzene and chromatographed on a column of alumina, 30 cm. x 2 cm. No separation into bands took place. On developing with benzene the total amount was eluted. The filtrate was collected in four portions, each of which was

evaporated to dryness. The melting points of each residue were approximately the same as the original, indicating that no separation had taken place.

(c) The reaction was again repeated, but this time mercuric sulphate was used instead of the oxide, and the mixture was stirred mechanically throughout the 4½ hours. The product obtained after chlorination was again found to be 1:5-dichloroanthraquinone.

The first filtrate, which had previously been discarded in accordance with the instructions given by Goldberg, was neutralised with caustic soda and evaporated to about $^1/_3$ volume. On cooling, a yellow precipitate was obtained, which was partially composed of sodium sulphate crystals. It was filtered, drained, and extracted with hot alcohol. The yellow alcoholic solution was evaporated. The yield was 6 g., which on chlorination as formerly gave 2.5 g. of product.

m.p. - 158° - 165°.

Repeated crystallisations from benzene and glacial acetic acid did not purify the substance materially, and the highest melting point obtained was 178° - 190°.

(d) A further attempt was made with twice the amount of fuming sulphuric acid (20% SO_3) and again with mercuric sulphate.

After heating at 160° with constant stirring for 4½ hours, half the reaction mixture was removed, and the remainder was allowed to stay

at the same temperature for a further 4½ hours.

(i) The portion withdrawn was cooled, and poured into 600 ml. cold water. A dark solution was formed, with only a trace of insoluble matter. After filtering, it was boiled with a few millilitres of concentrated nitric acid, but the colour did not become any paler. A solution of 40 g. sodium hydroxide in 50 ml. water was added, and a purple colour was produced. The solution was boiled until its volume was reduced to about 150 ml. On cooling a semi-solid mass formed which would not filter. Sufficient dilute sulphuric acid was added to change the colour to brown, and filtration was then possible.

Half of the brown precipitate was dissolved in 800 ml. water, filtered through charcoal, and chlorinated by boiling with 20 g. potassium chlorate and 90 ml. concentrated hydrochloric acid. The dark brown colour faded when the acid was added, and the solution became pale yellow when chlorine began to be evolved.

Yield of chlorinated compound 2.5 g.

- m.p. 175° - 230°.

Crystallised from glacial acetic acid

- m.p. 218° - 245°.

Recrystallised - m.p. 250° - 254°.

Mixed m.p. with 1:5-dichloroanthraquinone 214° - 240°. Yield 0.2 g.

Analysis: Found: Chlorine = 31.65%.

Calc. for $C_{14}H_5O_2Cl_3$: Chlorine = 34.19%.

Calc. for $C_{14}H_6O_2Cl_2$: Chlorine = 25.63%.

The purple colour with excess caustic soda, and the change of colour on acidification, suggested the possibility of hydroxy-compounds being present. The remainder of the brown precipitate was dried, and boiled for ½ hour with 20 ml. acetic anhydride containing 4 drops concentrated sulphuric acid. A pale yellow solution resulted, but on dilution with water, no precipitate was formed.

(ii) The remainder of the reaction mixture, which had been heated for 9 hours, was cooled and poured into 750 ml. water. It was filtered from the small amount of insoluble matter, and the filtrate boiled with a few millilitres of concentrated nitric acid. 50 g. sodium hydroxide dissolved in 100 ml. water were added. The colour remained yellowish brown. The solution was boiled until it measured about 150 ml., and was then cooled. The yellow solid which separated was filtered off. It was then dissolved in the minimum amount of boiling water, cooled slightly, and filtered from the small precipitate formed. The filtrate was cooled and the crystals formed were removed by filtration. They were recrystallised from water. Yield - 15 g.

The product was chlorinated as formerly, and 4 g. of chlorinated substance was formed.

Repeated crystallisations from benzene, glacial acetic acid and light petroleum failed to purify the substance sufficiently to give a sharp melting point.

1.3 g. were dissolved in a mixture of light petroleum (100° - 120°) 1 part and benzene 2 parts, and was chromatographed on a column of alumina 55 cm. x 2.5 cm. The column was developed with the same solvent and the filtrate was collected in 200 ml. portions. The first four portions on evaporation gave residues with m.p. 170° - 200°. The next three portions showed a distinct rise in melting points - 230° - 250°. The residues from these three portions when mixed and crystallised from benzene and light petroleum gave pale yellow crystals, m.p. 247° - 250°. Mixed m.p. with 1:5-dichloroanthraquinone showed no depression.

(e) The experiment was repeated using the original quantity of fuming sulphuric acid, but increasing the time of heating to 9 hours. The product was worked up in the usual way. The chlorinated product (5 g.) had a melting point of 158° - 185°, but crystallisation from several solvents failed to raise it.

(f) The same quantities as used in (a) were treated as described by Goldberg, but on this occasion care was taken to powder the red mercuric oxide finely, and triturate it with the fuming sulphuric acid before transferring it to the flask. The mixture was shaken and stirred

occasionally during the 4½ hours heating. On working up and chlorinating as before, the product was again found to be 1:5-dichloroanthraquinone.

Attempted preparation of 1:4:5-trichloroanthraquinone from 1:5-dichloroanthraquinone.

1:5-dichloroanthraquinone (50 g.) was sulphonated by the method described by Goldberg (56). On evaporating the solution to small bulk and cooling the precipitate formed was dark brown. The filtrate was yellow - it was concentrated and cooled. Yellow plates were obtained which were dissolved in the minimum amount of boiling water, and a saturated solution of sodium chloride (5 g.) added. On standing overnight 4 g. of yellow crystals were obtained. On chlorinating these as described, no yield was obtained after 8 hours boiling.

Another attempt was made using 6 g. red mercuric oxide as catalyst. 43 g. of a yellow product was obtained. 10 g. of this was chlorinated as formerly yielding 5.5 g. pale yellow crystals, m.p. over 300°. Crystallised from benzene.

Analysis: Found: Chlorine = 40.8%.

Calc. for $C_{14}H_4O_2Cl_4$: Chlorine = 41.04%.

The tetrachloro- derivative has thus been obtained.

Preparation of 1:4:5-trichloroanthraquinone from 1:8-dichloroanthraquinone.

1:8-dichloroanthraquinone (20 g.) was sulphonated as described by Goldberg. 3.5 g. sulphonated product was obtained which was

chlorinated as formerly.

Yield - 2 g. yellow crystals. m.p. 252° - 256°.

Crystallised from benzene and light petroleum - m.p. 258° - 261°.

Recrystallised from glacial acetic acid - m.p. 263° - 265°. (lit. 254°.)

Analysis: Found: Chlorine = 34.7%.

Calc. for $C_{14}H_5O_2Cl_3$: Chlorine = 34.19%.

Replacement of chlorine by hydroxyl in 1:4:5-trichloroanthraquinone.

1 g. of 1:4:5-trichloroanthraquinone was treated with calcium hydroxide, copper and water by heating at 210° - 215° for 24 hours, and worked up as before.

Crude yield = 0.65 g.

This was dissolved in warm solution of caustic soda, filtered and acidified with dilute hydrochloric acid. 0.55 g. alkali soluble red powder was obtained, m.p. 200° - 215°. Crystallisation from glacial acetic acid and then from benzene gave red needles which had a constant m.p. 267° - 268°.

There was no change in melting point when this substance was mixed with the crystals obtained when the chlorine in 1:4:5:8-tetrachloroanthraquinone was replaced with hydroxyl (see page 112).

Preparation of toluhydroquinone.

Toluquinone (25 g.) was suspended in about 500 ml. water, and sulphur dioxide passed through the suspension until solution was complete. The solution after darkening deposited a dark brown

precipitate. Passing of sulphur dioxide was continued until the precipitate redissolved, forming a pale yellow solution which smelt strongly of sulphur dioxide. The solution was extracted with ether in a separating funnel; the ethereal solution was dried over fused calcium chloride and evaporated. Yield - 18 g. m.p. - 125° - 130°. Crystallisation from benzene produced colourless plates, m.p. 127° - 128°.

Attempted preparation of 1:4:5:8-tetrahydroxy-3-methylanthraquinone.

Toluhydroquinone	11.5 g.
3:6-dichlorophthalic anhydride	30 g.
Boric Acid Crystals	5 g.
95% Sulphuric Acid	200 g.

A mixture of the above substances was treated by the method described by Bigelow and Reynolds (22) for the preparation of quinizarin. The mixture, at first fluid, gradually thickened and evolved sulphur dioxide. After heating in an oil bath at 200° for 3½ hours and cooling, the product was a black porous solid which broke up easily. It was boiled twice with 500 ml. water, and the washings (which were colourless) discarded. The solid was added to a litre of boiling water and 35 ml. 10N caustic potash solution added slowly. Very little of the solid went into solution, even on boiling for a few minutes. On filtering a red filtrate was obtained. On acidification with hydrochloric acid the filtrate was decolourised, but the precipitate was negligible.

The solid was insoluble in acetone, benzene,

glacial acetic acid, alcohol and pyridine. It did not sublime, and did not melt in the bunsen flame. An attempt to acetylate with acetic anhydride was unsuccessful. A portion was extracted for 2 hours with glacial acetic acid in a Soxhlet apparatus. On evaporation of the solvent a small white residue was obtained which was identified as boric acid.

The product appeared to consist mainly of carbon.

Attempted preparation of 1:4:5:8-tetrahydroxyanthraquinone.

Hydroquinone (11.5 g.) was treated by the method described in the previous experiment. The caustic potash solution was purple. On acidifying with hydrochloric acid and filtering, 1.4 g. of a red solid was obtained, m.p. over 300°.

Crystallisation from benzene gave bronze coloured needles, which were crimson when powdered.

m.p. over 300°.

Analysis: Found: C = 56.16%; H = 3.01%; Cl = 5.87%.

Calc.for $C_{14}H_8O_6$: C = 61.8%; H = 2.9%.

Calc.for $C_{14}H_7O_5Cl$: C = 57.84%; H = 2.41%; Cl = 12.22%.

It is probable that impure tetrahydroxyanthraquinone has been formed.

A small amount was acetylated with acetic anhydride in the usual way. A yellowish brown powder was obtained, m.p. 245° - 250° (decomposed). The amount obtained was so small that crystallisation was not possible.

SUMMARY.

1. A preliminary examination of extracts of senna by chromatography showed that alumina is not suitable as an adsorbent owing to the formation of lakes with the hydroxyanthraquinones. "Blocking" of the hydroxyl groups by acetylation gives improved results. Heavy magnesium carbonate shows possibilities of being a suitable adsorbent.

2. An investigation of the methods of reducing anthraquinones to anthrones has been carried out. The use of tin, glacial acetic acid and fuming hydrochloric acid was found to be most successful.

3. Anthrones were found to undergo aerial oxidation in presence of traces of alkali. This may occur on a chromatographic column.

4. The synthesis of chloroanthraquinones from chlorophthalic anhydrides and phenols has been investigated.

5. The replacement of chlorine by hydroxyl was carried out in a series of α-chloroanthraquinones. Consistent results were obtained by heating the chloroanthraquinones under pressure with calcium hydroxide and water.

In conclusion, the author wishes to express his appreciation of the privilege he has enjoyed in working under the supervision of Dr. Neil Campbell. Dr. Campbell's ever available advice and constant encouragement have been of great value throughout the period of this research, and very sincere thanks are given to him.

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